(19) 대한민국특허청(KR)
(12) 등록특허공보(B1)

(45) 공고일자 2024년11월14일
(11) 등록번호 10-2729768
(24) 등록일자 2024년11월08일

(51) 국제특허분류(Int. Cl.)
C12N 15/90 (2006.01) *C12N 15/86* (2006.01)
C12N 9/10 (2006.01)
(52) CPC특허분류
C12N 15/907 (2013.01)
C12N 15/86 (2013.01)
(21) 출원번호 10-2020-7013411
(22) 출원일자(국제) 2018년10월11일
심사청구일자 2021년10월08일
(85) 번역문제출일자 2020년05월11일
(65) 공개번호 10-2020-0064129
(43) 공개일자 2020년06월05일
(86) 국제출원번호 PCT/US2018/055412
(87) 국제공개번호 WO 2019/075200
국제공개일자 2019년04월18일
(30) 우선권주장
62/571,672 2017년10월12일 미국(US)
(뒷면에 계속)
(56) 선행기술조사문헌
WO2000071701 A1
(뒷면에 계속)
전체 청구항 수 : 총 19 항

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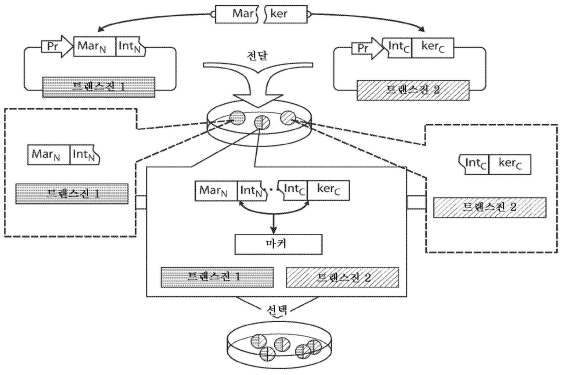
(54) 발명의 명칭 **트랜스제닉 선택 방법 및 조성물**

(57) 요 약

본 개시내용은 트랜스제닉 세포의 생성 및 선택을 위한 분할 인테인 선택가능한 마커 시스템을 제공한다.

대 표 도



(52) CPC특허분류
C12N 9/1029 (2013.01)
C07K 2319/60 (2013.01)
C07K 2319/60 (2013.01)
C07K 2319/92 (2013.01)
C12N 2740/16043 (2013.01)

(56) 선행기술조사문헌
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ACS Synth Biol.,1(10):445-450(2012.9.4.)
미국 특허공보 US6858775(2005.2.22.)*
Biotechnology Letters.,37(11):2121-2137(2015.7.8.)
KR1020140069313 A
*는 심사관에 의하여 인용된 문헌

(30) 우선권주장
62/608,478 2017년12월20일 미국(US)
62/616,281 2018년01월11일 미국(US)
62/624,629 2018년01월31일 미국(US)

명 세 서

청구범위

청구항 1

(a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 제1 트랜스진을 포함하는 제1 벡터; 및

(b) (i) 선택가능한 마커 단백질의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 제2 트랜스진을 포함하는 제2 벡터

를 진핵생물 세포로 전달하는 것을 포함하는 방법이며,

여기서 제1 트랜스진은 제2 트랜스진과 상이하고,

제1 트랜스진은 관심있는 제1 분자를 코딩하고, 제2 트랜스진은 관심있는 제1 분자와 상이한 관심있는 제2 분자를 코딩하고,

인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 N-말단 단편 및 C-말단 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성하고,

단일 진핵생물 세포는 전장 선택가능한 마커 단백질, 관심있는 제1 분자 및 관심있는 제2 분자를 발현하는 것인 방법.

청구항 2

제1항에 있어서, 트랜스제닉 진핵생물 세포를 생성하기 위해 진핵생물 세포로의 제1 및 제2 벡터의 도입을 허용하는 조건 하에 진핵생물 세포를 유지하는 것을 추가로 포함하는 방법.

청구항 3

제2항에 있어서, 전장 선택가능한 마커 단백질을 포함하는 트랜스제닉 진핵생물 세포를 선택하는 것을 추가로 포함하는 방법.

청구항 4

제1항 내지 제3항 중 어느 한 항에 있어서, 진핵생물 세포가 포유동물 세포인 방법.

청구항 5

제1항 내지 제3항 중 어느 한 항에 있어서, 선택가능한 마커 단백질이 항생제 내성 단백질인 방법.

청구항 6

제5항에 있어서, 항생제 내성 단백질이 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1 또는 블라스티시딘에 대한 내성을 부여하는 것인 방법.

청구항 7

제5항에 있어서, 항생제 내성 단백질이 hygB 유전자, bsr 유전자, pac 유전자 또는 neo 유전자에 의해 코딩된 것인 방법.

청구항 8

제1항 내지 제3항 중 어느 한 항에 있어서, 선택가능한 마커 단백질이 형광 단백질인 방법.

청구항 9

제8항에 있어서, 형광 단백질이 TagCFP, mTagCFP2, Czurite, ECFP2, mKalama1, Sirius, Sapphire, T-Sapphire,

ECFP, Cerulean, SCFP3C, mTurquoise, mTurquoise2, 모노머 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, 모노머 Czami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, 모노머 Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mScarlet, mTangerine, tdTomato, TagRFP, TagRFP-T, mCpple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, TagRFP657, IFP1.4 및 iRFP로부터 선택된 것인 방법.

청구항 10

제1항 내지 제3항 중 어느 한 항에 있어서, 인테인이 분할 인테인인 방법.

청구항 11

제10항에 있어서, 분할 인테인이 자연 분할이며, 임의로 여기서 자연 분할 인테인이 DnaE 인테인으로부터 선택되고, 임의로 DnaE 인테인이 시네코시스티스(*Synechocystis*) sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메(*Nostoc punctiforme*) (NpuDnaE) 인테인으로부터 선택된 것인 방법.

청구항 12

제10항에 있어서, 분할 인테인이 조작된 분할 인테인이며, 임의로 여기서 조작된 분할 인테인이 DnaB 인테인 또는 GyrB 인테인으로부터 조작되고, 임의로 조작된 분할 인테인이 SspDnaB S1 인테인 또는 SspGyrB S11 인테인인 방법.

청구항 13

제1항 내지 제3항 중 어느 한 항에 있어서, 관심있는 제1 및/또는 제2 분자가 단백질 또는 비-코딩 리보핵산(RNA)이고, 임의로 여기서 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 방법.

청구항 14

제1항 내지 제3항 중 어느 한 항에 있어서, 제1 및/또는 제2 벡터가 플라스미드 벡터 또는 바이러스 벡터인 방법.

청구항 15

(a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 제1 트랜스진을 포함하는 제1 벡터; 및

(b) (i) 선택가능한 마커 단백질의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 제2 트랜스진을 포함하는 제2 벡터

를 포함하는 진핵생물 세포이며,

여기서 제1 트랜스진은 제2 트랜스진과 상이하고,

제1 트랜스진은 관심있는 제1 분자를 코딩하고, 제2 트랜스진은 관심있는 제1 분자와 상이한 관심있는 제2 분자를 코딩하고,

인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 N-말단 단편 및 C-말단 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성하는 것인,

전장 선택가능한 마커 단백질, 관심있는 제1 분자 및 관심있는 제2 분자를 발현하는 진핵생물 세포.

청구항 16

(a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 제1 트랜스진을 포함하는 제1 벡터,

(b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질

의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 제2 트랜스진을 포함하는 제2 벡터, 및

(c) (i) 선택가능한 마커 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 제3 트랜스진을 포함하는 제3 벡터

를 진핵생물 세포로 전달하는 것을 포함하는 방법이며,

여기서 제1 트랜스진은 제2 트랜스진과 상이하고, 제1 트랜스진은 제3 트랜스진과 상이하고, 제2 트랜스진은 제3 트랜스진과 상이하고,

제1 트랜스진은 관심있는 제1 분자를 코딩하고, 제2 트랜스진은 관심있는 제2 분자를 코딩하고, 제3 트랜스진은 관심있는 제3 분자를 코딩하고,

관심있는 제1 분자는 관심있는 제2 분자와 상이하고, 관심있는 제1 분자는 관심있는 제3 분자와 상이하고, 관심있는 제2 분자는 관심있는 제3 분자와 상이하고,

제1 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 중심 단편으로의 선택가능한 마커 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 C-말단 단편으로의 선택가능한 마커 단백질의 중심 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성하고,

단일 진핵생물 세포는 전장 선택가능한 마커 단백질, 관심있는 제1 분자, 관심있는 제2 분자 및 관심있는 제3 분자를 발현하는 것인 방법.

청구항 17

제16항에 있어서, 트랜스제닉 진핵생물 세포를 생성하기 위해 진핵생물 세포로의 제1, 제2 및 제3 벡터의 도입을 허용하는 조건 하에 진핵생물 세포를 유지하는 것을 추가로 포함하는 방법.

청구항 18

제17항에 있어서, 전장 선택가능한 마커 단백질을 포함하는 트랜스제닉 진핵생물 세포를 선택하는 것을 추가로 포함하는 방법.

청구항 19

(a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 제1 트랜스진을 포함하는 제1 벡터,

(b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 제2 트랜스진을 포함하는 제2 벡터, 및

(c) (i) 선택가능한 마커 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 제3 트랜스진을 포함하는 제3 벡터

를 포함하는 진핵생물 세포이며,

여기서 제1 트랜스진은 제2 트랜스진과 상이하고, 제1 트랜스진은 제3 트랜스진과 상이하고, 제2 트랜스진은 제3 트랜스진과 상이하고,

제1 트랜스진은 관심있는 제1 분자를 코딩하고, 제2 트랜스진은 관심있는 제2 분자를 코딩하고, 제3 트랜스진은 관심있는 제3 분자를 코딩하고,

관심있는 제1 분자는 관심있는 제2 분자와 상이하고, 관심있는 제1 분자는 관심있는 제3 분자와 상이하고, 관심있는 제2 분자는 관심있는 제3 분자와 상이하고,

제1 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 중심 단편으로의 선택가능한 마커 단백

질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 C-말단 단편으로의 선택가능한 마커 단백질의 중심 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성하는 것인,

전장 선택가능한 마커 단백질, 관심있는 제1 분자, 관심있는 제2 분자 및 관심있는 제3 분자를 발현하는 진핵생물 세포.

청구항 20

삭제

청구항 21

삭제

발명의 설명

기 술 분 야

[0001] <관련 출원>

[0002] 본 출원은 35 U.S.C. § 119(e) 하에 2018년 1월 11일에 출원된 미국 가출원 번호 62/616,281, 2017년 12월 20일에 출원된 미국 가출원 번호 62/608,478, 2018년 1월 31일에 출원된 미국 가출원 번호 62/624,629, 2017년 10월 12일에 출원된 미국 가출원 번호 62/571,672의 이익을 주장하며, 이들은 그 전문이 본원에 참조로 포함된다.

[0003] <서열 목록>

[0004] 본 출원은 컴퓨터 판독가능한 형태의 서열 목록 (파일명: J022770007WO00-SEQ-HJD; 1.50 MB - ASCII 텍스트 파일; 2018년 10월 3일 생성됨)을 함유하며, 이는 그 전문이 본원에 참조로 포함되고 본 개시내용의 일부를 형성한다.

배 경 기 술

[0005] 선택가능한 마커는 원하는 유전자형을 갖는 조작된 세포를 선택하기 위한 트랜스제네시스(transgenesis) 및 게놈 편집에서 광범위하게 채택된다. 항생제 내성 유전자 (항생제 내성 단백질을 코딩함)는 특정 항생제에 대한 내성을 제공하여, 이들 내성 유전자를 발현하는 세포만이 생존하고 증식하도록 한다. 진핵생물 세포에 사용하기 위한 이용가능한 항생제 내성 유전자/항생제는 hygB/히그로마이신, neo/게네티신(Geneticin)®/G418, pac/퓨로마이신, Sh bla/플레오마이신 D1 (제오신(Zeocin)™), 및 bsd/블라스티시딘을 포함한다. 형광 단백질, 예컨대 녹색 형광 단백질(green fluorescent protein)(GFP)은 세포 선택의 또 다른 수단, 예를 들어 형광-활성화 세포 분류법(fluorescent-activated cell sorting)(FACS) 기술 또는 형광 현미경검사를 통한 수단을 제공한다.

발명의 내용

[0006] <요약>

[0007] 진핵생물 (예를 들어, 포유동물) 세포에 사용하기 위한 이용가능한 항생제 내성 유전자/항생제의 수가 제한되어 있으므로, 다중 트랜스진(transgene)을 함유하는 세포를 확인하기 위한 선택 방식이 제한된다. 진핵생물 세포에서 항생제 내성을 부여하는 별개의 유전자의 수가 제한되어 있을 뿐만 아니라, 최소 3개의 상이한 항생제 내성 유전자의 동시 사용은 트랜스제닉(transgenic) 세포의 건강에 유해한 영향을 미칠 수 있다. 항생제 선택은 연속적으로 수행될 수 있지만, 이 과정은 시간이 많이 걸린다. 트랜스제닉 세포를 확인하기 위한 선택 방식에 대한 이들 제한은 다중 트랜스진이 도입된 세포를 확인할 필요가 있는 경우 (예를 들어, 트랜스제닉 유기체, 예를 들어 동물 모델, 예컨대 마우스 모델을 생성하기 위해) 문제가 된다.

[0008] 예를 들어, 2개 이상의 트랜스진 (예를 들어, 이중-트랜스제닉, 삼중-트랜스제닉 등)을 보유하는 세포 및/또는 유기체의 생성 및/또는 확인에 유용한 방법, 조성물 및 키트가 본원에 제공된다. 예를 들어, 조성물 및 키트는 2, 3 또는 4개의 트랜스진을 보유하는 세포 및/또는 유기체의 생성 및/또는 확인을 위해 사용될 수 있다. 이 기술은 적어도 부분적으로, 인테인(intein) 자동-프로세싱 도메인에 의해 개시된 단백질 스플라이싱 메커니즘에

기초하며, 이는 다중 (예를 들어, 2, 3 또는 4개) 별도의 선택가능한 마커 단백질 단편의 다중-트랜스제닉 세포 (이중-트랜스제닉 세포, 삼중-트랜스제닉 세포 또는 사중-트랜스제닉 세포)에서 특이적 연결 (접합)을 용이하게 한다. 다중-트랜스제닉 세포에서 2, 3, 4개 또는 그 이상의 별도의 선택가능한 마커 단백질 단편의 연결은 예를 들어, 항생제 내성 (항생제 내성 단백질)을 부여하거나 적절한 파장의 빛 하에 형광 (형광 단백질)을 낼 수 있는 전장 선택가능한 마커 단백질을 생성한다. 전장 항생제 내성 유전자를 발현하는 세포는 상응하는 항생제의 존재 하에 생존하므로, 다중-트랜스제닉 (예를 들어, 이중-트랜스제닉, 삼중-트랜스제닉 또는 사중-트랜스제닉) 세포로서 선택된다. 마찬가지로, 전장 기능성 형광 단백질을 발현하는 세포는 적절한 파장의 빛 하에 형광을 내므로, 다중-트랜스제닉 (예를 들어, 이중-트랜스제닉, 삼중-트랜스제닉 또는 사중-트랜스제닉) 세포로서 선택된다.

[0009] 그러므로, 일부 실시양태에서, 본 개시내용은 2종 이상의 벡터를 진핵생물 세포를 포함하는 조성물로 전달하는 것을 포함하는 방법을 제공하며, 여기서 각각의 벡터는 (i) N-말단 인테인 단백질 단편 및/또는 C-말단 인테인 단백질 단편에 연결된 선택가능한 마커 단백질 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 분자를 코딩하는 뉴클레오티드 서열을 포함하며, 여기서 인테인 단백질 단편은 전장 기능 단백질을 형성하도록 프레임에 연결된 경우, 선택가능한 마커 단백질 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성한다. 예를 들어, 2종의 벡터가 세포의 집단으로 전달된 경우 (예를 들어, 형질감염 조건 하에), 일부 세포는 제1 벡터를 수용할 것이고 (벡터가 세포에 도입됨), 일부 세포는 제2 벡터를 수용할 것이고, 일부 세포는 2종의 벡터 모두를 수용할 것이다. 2종의 벡터 모두를 수용하는 세포만이 전장 기능성 선택가능한 마커 단백질을 발현할 수 있으므로, 이들 세포만이 이중-트랜스제닉 세포로서 선택된다.

[0010] 일부 실시양태에서, (a) (i) N-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 선택가능한 마커 단백질 단편 (예를 들어, 항생제 내성 단백질 단편 또는 형광 단백질 단편)을 코딩하는 뉴클레오티드 서열 및 (ii) 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터, 및 (b) (i) 제2 선택가능한 마커 단백질 단편 (예를 들어, 항생제 내성 단백질 단편 또는 형광 단백질 단편)으로부터 상류에 있는, C-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터를 진핵생물 세포를 포함하는 조성물로 전달하는 것을 포함하며, 여기서 N-말단 인테인 단백질 단편 및 C-말단 인테인 단백질 단편은 제2 선택가능한 마커 단백질 단편으로의 제1 선택가능한 마커 단백질 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성하는 것인 방법이 본원에 제공된다. 2종의 벡터가 세포의 집단으로 전달된 경우 (예를 들어, 형질감염 조건 하에), 일부 세포는 제1 벡터를 수용할 것이고 (벡터가 세포에 도입됨), 일부 세포는 제2 벡터를 수용할 것이고, 일부 세포는 2종의 벡터 모두를 수용할 것이다. 2종의 벡터 모두를 수용하는 세포만이 전장 기능성 선택가능한 마커 단백질을 발현할 수 있으므로, 이들 세포만이 이중-트랜스제닉 세포로서 선택된다.

[0011] 다른 실시양태에서, 방법은 (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질 (예를 들어, 항생제 내성 단백질 또는 형광 단백질)의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터, (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및 (c) (i) 선택가능한 마커 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 중심 단편으로의 선택가능한 마커 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 C-말단 단편으로의 선택가능한 마커 단백질의 중심 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성한다. 3종의 벡터가 세포의 집단으로 전달된 경우 (예를 들어, 형질감염 조건 하에), 일부 세포는 제1 벡터를 수용할 것이고 (벡터가 세포에 도입됨), 일부 세포는 제2 벡터를 수용할 것이고, 일부 세포는 제3 벡터를 수용할 것이고, 일부 세포는 2종의 상이한 벡터를 수용할 것이고, 일부 세포는 3종의 벡터 모두를 수용할 것이다. 3종의 벡터 모두를 수용하는 세포만이 전장 기능성 선택가능한 마커 단백질을 발현할 수 있으므로, 이들 세포만이 삼중-트랜스제닉 세포로서 선택된다.

[0012] 또 다른 실시양태에서, 방법은 (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질 (예를 들어, 항생제 내성 단백질 또는 형광 단백질)의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터, (b) (i) 제

2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 제1 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, (c) (i) 제3 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 제2 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터, 및 (d) (i) 선택가능한 마커 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제3 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제4 벡터를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 제1 중심 단편으로의 선택가능한 마커 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 제2 중심 단편으로의 선택가능한 마커 단백질의 제1 중심 단편의 연결을 촉매하고, 제3 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 C-말단 단편으로의 선택가능한 마커 단백질의 제2 중심 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성한다. 4종의 벡터가 세포의 집단으로 전달된 경우 (예를 들어, 형질감염 조건 하에), 일부 세포는 제1 벡터를 수용할 것이고 (벡터가 세포에 도입됨), 일부 세포는 제2 벡터를 수용할 것이고, 일부 세포는 제3 벡터를 수용할 것이고, 일부 세포는 제4 벡터를 수용할 것이고, 일부 세포는 2종의 상이한 벡터를 수용할 것이고, 일부 세포는 3종의 상이한 벡터를 수용할 것이고, 일부 세포는 4종의 벡터 모두를 수용할 것이다. 4종의 벡터 모두를 수용하는 세포만이 전장 기능성 선택가능한 마커 단백질을 발현할 수 있으므로, 이들 세포만이 사중-트랜스제닉 세포로서 선택된다.

[0013] 명세서의 예들 또는 하나의 섹션에만 개시된 것들을 포함하여 본원에 기재된 임의의 한 실시양태는 명백하게 부인되지 않는 한 임의의 하나 이상의 다른 실시양태와 조합될 수 있는 것으로 의도되는 것으로 이해되어야 한다.

도면의 간단한 설명

[0014] 도 1a-1b. 2종의 별도의 트랜스제닉 벡터의 항생제 공동선택을 위한 분할 선택가능한 마커. (도 1a) 선택가능한 마커의 코딩 서열은 N-말단 단편 (MarN) 및 C-말단 단편 (kerC)으로 분할되고, 분할 인테인의 N-말단 단편 (IntN)의 상류 및 분할 인테인의 C-말단 단편 (IntC)의 하류 각각에 별도로 클로닝되며, 2종의 상이한 벡터 각각은 상이한 트랜스진을 보유한다. 이들 벡터는 세포로 전달되어 벡터 중 하나 또는 벡터 중 둘 모두를 함유하는 세포의 하위집단을 수득한다. 2종의 인테인-분할 선택가능한 마커 단편 ("마커트론(markertron)")을 발현하는 두 벡터 모두를 갖는 세포에서만 단백질 트랜스-스플라이싱을 수행하여 전장 선택가능한 마커를 재구성하여 이중 트랜스제닉 세포의 특이적 선택 및 풍부를 허용한다. (도 1b) 항생제 내성 유전자에 대한 인테인과 호환가능한 분할점을 스크리닝하기 위해, 본 발명자들은 시험된 인테인의 유형에 대한 이음부 요건에 따라 잠재적인 분할점을 확인한 후, TagBFP2 또는 mCherry 형광 단백질이 장착된 렌티바이러스 벡터 상의 분할 인테인 스캐폴드(scaffold)에 상응하는 N-말단 및 C-말단 단편을 클로닝하였으며, 이는 선택 효율을 평가하기 위한 시험 트랜스진으로서 작용한다. 이들은 렌티바이러스 형질도입을 통해 세포로 전달된다. 그 후, 세포는 복제 플레이트로 분할되었으며, 하나는 항생제 선택을 거친 반면, 다른 하나는 비-선택 배지에서 유지되었다. 항생제 선택 후, 복제 배양물을 유동 세포계측법에 의해 분석하였다.

도 2a-2f. 인테인-분할 내성 (Intres) 유전자 (선택가능한 마커 유전자라고도 함) 및 플라스미드의 분할점에 대한 세부사항. (도 2a) 히그로마이신 내성 단백질 (서열식별번호(SEQ ID NO): 1)에 대한 분할점. 히그로마이신 내성 단백질의 아미노산 서열에는 본 연구에서 특징화된 분할점을 표지하는 클라우드(cloud)가 제공된다. 표지 내에서, 상단 행은 표 1에 상응하는 플라스미드 번호를 나타낸다. 하단 행은 N-말단 단편에서 마지막 아미노산의 잔기 수, 사용된 인테인의 종, 및 C-말단 단편에서 첫 번째 아미노산의 잔기 수를 나타낸다. "^C"는 시스테인의 삽입을 나타낸다. (도 2b) 퓨로마이신 내성 단백질 (서열식별번호: 2)에 대한 분할점. 퓨로마이신 내성 단백질의 아미노산 서열에는 본 연구에서 특징화된 분할점을 표지하는 클라우드가 제공된다. 표지 내에서, 상단 행은 표 1에 상응하는 플라스미드 번호를 나타낸다. 하단 행은 N-말단 단편에서 마지막 아미노산의 잔기 수, 사용된 인테인의 종, 및 C-말단 단편에서 첫 번째 아미노산의 잔기 수를 나타낸다. "^C"는 시스테인의 삽입을 나타낸다. (도 2c) 네오마이신 내성 단백질 (서열식별번호: 3)에 대한 분할점. 네오마이신 내성 유전자의 아미노산 서열에는 본 연구에서 특징화된 분할점을 표지하는 클라우드가 제공된다. 표지 내에서, 상단 행은 표 1에 상응하는 플라스미드 번호를 나타낸다. 하단 행은 N-말단 단편에서 마지막 아미노산의 잔기 수, 사용된 인테인의 종, 및 C-말단 단편에서 첫 번째 아미노산의 잔기 수를 나타낸다. (도 2d) 블라스티시딘 내성 단백질 (서열식별번호: 4)에 대한 분할점. 블라스티시딘 내성 유전자의 아미노산 서열에는 본 연구에서 특징화된 분할점을 표지하는 클라우드가 제공된다. 표지 내에서, 상단 행은 표 1에 상응하는 플라스미드 번호

를 나타낸다. 하단 행은 N-말단 단편에서 마지막 아미노산의 잔기 수, 사용된 인테인의 종, 및 C-말단 단편에서 첫 번째 아미노산의 잔기 수를 나타낸다. (도 2e) 녹색 형광 단백질 (서열식별번호: 5)에 대한 분할점. (도 2f) mScarlet 형광 단백질 (서열식별번호: 6)에 대한 분할점. mScarlet 유전자의 아미노산 서열에는 본 연구에서 특징화된 분할점을 표지하는 클라우드가 제공된다. 표지 내에서, 상단 행은 표 1에 상응하는 플라스미드 번호를 나타낸다. 하단 행은 N-말단 단편에서 마지막 아미노산의 잔기 수, 사용된 인테인의 종, 및 C-말단 단편에서 첫 번째 아미노산의 잔기 수를 나타낸다. "^C"는 시스테인의 삽입을 나타낸다.

도 3. 2-마커트론 히그로마이신 (Hygro) 인테인-분할 내성 (Intres) 유전자. 상단 개략도는 히그로마이신 내성 유전자에 대해 시험된 분할점을 나타낸다. N-말단 단편의 마지막 잔기는 롤리팝(lollipop)의 상단 위에 표시되어 있다. 원형 롤리팝은 NpuDnaE 인테인을 사용하는 분할점을 나타내고, 사각형 롤리팝은 SspDnaB 인테인을 사용하는 분할점을 나타낸다. 줄 그어진 및 음영처리된 롤리팝은 세포에 히그로마이신 내성을 부여하지 못한 분할 쌍을 나타낸다. 아래의 컬럼 플롯은 유동 세포계측법에 의해 분석된 비-선택된 (백색 컬럼) 및 선택된 배양물 (파란색 컬럼) 중 이중-트랜스제닉 세포 (BFP+ mCherry+)의 백분율을 나타낸다.

도 4. 2-마커트론 퓨로마이신 (Puro) Intres 유전자. 상단 개략도는 퓨로마이신 내성 유전자에 대해 시험된 분할점을 나타내는 반면, 하단 컬럼 플롯은 비-선택된 (백색 컬럼) 및 선택된 배양물 (갈색 컬럼) 중 이중 트랜스제닉 세포의 백분율을 나타낸다.

도 5. 2-마커트론 네오마이신 (Neo) 내성 유전자. 상단 개략도는 네오마이신 내성 유전자에 대해 시험된 분할점을 나타내는 반면, 하단 컬럼 플롯은 비-선택된 (백색 컬럼) 및 선택된 배양물 (오렌지색 컬럼) 중 이중 트랜스제닉 세포의 백분율을 나타낸다.

도 6. 2-마커트론 블라스티시딘 (Blast) Intres 유전자. 상단 개략도는 블라스티시딘 내성 유전자에 대해 시험된 분할점을 나타내는 반면, 하단 컬럼 플롯은 비-선택된 (백색 컬럼) 및 선택된 (시안색 컬럼) 배양물 중 이중 트랜스제닉 세포의 백분율을 나타낸다.

도 7a-7c. 2-마커트론 Intres 마커를 갖는 게이트웨이-호환가능한(Gateway-compatible) 렌티바이러스 목적지 벡터. (도 7a) 각각의 분할 Intres 마커에 대한 게이트웨이-호환가능한 렌티바이러스 목적지 벡터 키트는 N-벡터 및 C-벡터로 이루어진다. N-벡터는 바이러스 LTR, CAGGS 프로모터, 게이트웨이 목적지 카세트 AttL, ccdB 유전자, 트랜스진을 보유하는 게이트웨이 공여자 벡터의 LR 클로나제-매개 재조합을 허용하는 클로람페니콜 내성 유전자, 이어서 N-마커트론의 다중시스트론(polycistronic) 발현을 허용하는 내부 리보솜 진입 부위 (IRES)를 함유한다. 유사하게, C-벡터는 C-마커트론을 함유하고, 또 다른 트랜스진의 재조합을 허용한다. (도 7b) TagBFP2 (트랜스진 1로서) 및 mCherry (트랜스진 2로서)는 게이트웨이 재조합에 의해 2-마커트론 Intres 플라스미드로 클로닝되고, 렌티바이러스 형질도입에 의해 세포로 전달되고, 이어서 항생제 선택 및 유동 세포계측법 분석이 수행되었다. 컬럼 플롯은 2-마커트론 히그로마이신 (Hygro, 파란색 컬럼), 퓨로마이신 (Puro, 갈색 컬럼), 및 네오마이신 (Neo, 오렌지색 컬럼) 실험으로부터의 선택된 배양물 대 그의 상응하는 비-선택 배양물 (백색 컬럼) 중 BFP+mCherry+ 이중-양성 세포의 백분율을 나타낸다. (도 7c) 핵을 GFP 형광으로 표지하는 NLS-GFP (트랜스진 1로서) 및 F-액틴을 mScarlet 형광으로 표지하는 lifeAct-mScarlet (트랜스진 2로서)은 전체 비-분할 히그로마이신 내성 유전자를 발현하는 렌티바이러스 벡터 또는 2-마커트론 히그로마이신 Intres 유전자를 갖는 렌티바이러스 벡터로 재조합되고, 이중-표지 세포를 제작하기 위해 U2OS 세포를 형질도입하는데 사용되었다. 대표적인 형광 현미경 이미지는 2주 동안 히그로마이신 선택 후 GFP, mScarlet 및 세포의 병합된 채널을 나타낸다.

도 8a-8c. 2종의 별도의 트랜스제닉 벡터의 형광-매개 공동선택을 위한 분할 mScarlet. (도 8a) 2-마커트론 mScarlet 단백질. 상단 개략도는 mScarlet에 대해 시험된 분할점을 나타낸다. N-말단 단편의 마지막 잔기는 롤리팝의 상단 위에 표시되어 있다. (도 8b) mScarlet에 대한 NpuDnaE 인테인-호환가능한 분할점을 스크리닝하기 위해, 본 발명자들은 NpuDnaE 인테인에 대한 이음부 요건에 따라 잠재적인 분할점을 확인한 후, TagBFP2 또는 EGFP 형광 단백질이 장착된 렌티바이러스 벡터 상의 분할 인테인 스캐폴드에 상응하는 N-말단 및 C-말단 단편을 클로닝하였으며, 이는 선택 효율을 평가하기 위한 시험 트랜스진으로서 작용한다. 이들은 렌티바이러스 형질도입을 통해 세포로 전달된다. 두 렌티바이러스 모두를 갖는 세포는 전장 mScarlet 형광 단백질을 재구성할 뿐만 아니라 TagBFP2 및 EGFP 트랜스진 둘 모두를 발현하기 위해 필수적인 단백질 스플라이싱 기구 및 mScarlet 단편을 함유한다. 세포에 FACS 분석을 수행하였다. 박스형 개략도는 플라스미드 쌍 33+34의 FACS 분석의 예를 나타낸다. P1 집단은 살아있는 단일항 세포에 대한 전방 산란 및 측방 산란을 위해 게이팅되었다. 이들로부터, 세포의 17.8%는 TagBFP 및 EGFP 트랜스진에 대해 이중 양성이다. P1 세포가 mScarlet-양성에 대해

추가로 게이팅된 경우 (mCherry 채널), 세포의 99.4%는 TagBFP 및 EGFP에 대해 이중 양성이다. (도 8c) 아래의 컬럼 플롯은 표시된 분할점 각각의 mScarlet-양성 세포의 백분율을 나타낸다. 위의 컬럼 플롯은 P1 세포 중 TagBFP+ EGFP+ 세포 (백색 컬럼) 및 P1 세포의 mScarlet-양성 서브세트 (적색 컬럼)의 백분율을 나타낸다.

도 9a-9d. 3종 이상의 트랜스제닉 벡터의 공동선택을 위한 다중-분할 선택가능한 마커. (도 9a) 선택가능한 마커는 3개의 단편 (M_1, M_2 및 M_3)으로 분배된다. 제1 마커 단편 (M_1)은 제1 분할 인테인의 N-말단 단편 (I_{N1})의 상류에 융합된다. 제2 마커 단편 (M_2)은 제1 분할 인테인의 C-말단 단편 (I_{C1})의 하류 및 제2 분할 인테인의 N-말단 단편 (I_{N2})의 상류에 융합된다. 제3 마커 단편 (M_3)은 제2 분할 인테인의 C-말단 단편 (I_{C2})의 하류에 융합된다. 제1 분할 인테인은 M_2로의 M1의 연결을 촉매하는 반면, 제2 분할 인테인은 M_3으로의 M_2의 연결을 촉매하여, 전체 선택가능한 마커를 효과적으로 재구성한다. (도 9b) "인테인 쇄" 메커니즘을 통한 k-분할 선택가능한 마커의 설계. 3-분할 시나리오와 유사하게, 선택가능한 마커는 k 단편으로 분배되고, 개입 분할 인테인에 의해 매개된 단백질 트랜스-스플라이싱을 통해 재구성된다. (도 9c) 2-분할 선택가능한 마커로부터 확인된 분할점은 3-분할 선택가능한 마커를 생성하기 위해 조합하여 사용되었다. 상응하는 단편은 렌티바이러스 벡터로 클로닝되어 벡터 당 3-분할 선택가능한 마커 구조 및 리포터 형광 트랜스진을 생성하였다. 그 후, 세포는 이들 벡터로부터 제조된 바이러스로 형질도입되었으며, 선택 또는 비-선택 배지로 분할되었다. 적절한 선택 기간 후, 배양물은 유동 세포계측법에 의해 분석되었다. (도 9d) 3-마커트론 히그로마이신 (Hygro) Intres. 상단 개략도는 히그로마이신 내성 유전자에 대해 시험된 분할점을 나타내며, N-말단 단편의 마지막 아미노산의 잔기 수는 NpuDnaE 및 SspDnaB 인테인을 각각 나타내는 원형 또는 사각형 롤리팝 위에 표시된다. 6종의 3-마커트론 히그로마이신 Intres를 시험하였으며, 각각은 각각의 경우에 사용된 2개의 분할점을 나타내는 원형 또는 사각형으로 넘버링된 라인으로 표시된다. 아래의 컬럼 플롯은 하기 숫자로 표시된 3-마커트론 히그로마이신 Intres에 대한 비-선택 (백색 컬럼) 및 선택 (파란색 컬럼) 배양물로부터의 삼중 트랜스제닉 (BFP+ GFP+ mCherry+) 세포의 백분율을 나타낸다.

도 10a-10c. 3-마커트론 히그로마이신 Intres 유전자를 갖는 게이트웨이-호환가능한 렌티바이러스 목적지 벡터. (도 10a) 바이러스 LTR, CAGGS 프로모터, 게이트웨이 목적지 카세트 AttL, ccdB 유전자, 트랜스진을 보유하는 게이트웨이 공여자 벡터의 LR 클로나제-매개 재조합을 허용하는 클로람페니콜 내성 유전자, 이어서 3종의 3-분할 히그로마이신 마커트론 각각의 다중시스트론 발현을 허용하는 내부 리보솜 진입 부위 (IRES)를 갖는 게이트웨이-호환가능한 렌티바이러스 목적지 벡터. (도 10b) TagBFP2 (트랜스진 1로서) 및 EGFP (트랜스진 2로서) 및 mCherry (트랜스진 3으로서)는 게이트웨이 재조합에 의해 3-분할 Intres 플라스미드로 클로닝되고, 렌티바이러스 형질도입에 의해 세포로 전달되고, 이어서 항생제 선택 및 유동 세포계측법 분석이 수행되었다. (도 10c) 컬럼 플롯은 선택된 히그로마이신 (파란색 컬럼) 대 그의 상응하는 비-선택 배양물 (백색 컬럼) 중 BFP+GFP+mCherry+ 삼중-양성 세포의 백분율을 나타낸다.

도 11. 4-분할 Hygro intres. (a) 4종의 상이한 플라스미드에 의해 발현된 4-분할 hygro intres의 마커트론. 플라스미드 115는 히그로마이신 내성 유전자의 아미노산 1~89 [Hygro(1-89)]를 NpuDnaE(N) 및 류신 지퍼 A 모티프 (LZA)로 융합함으로써 생성된 마커트론을 발현한다. 플라스미드 116은 N-말단으로부터 C-말단으로 류신 지퍼 B 모티프 (LZB)-NpuDnaGEP(C), Hygro(90-200) 및 SspDnaB(N)를 융합함으로써 생성된 마커트론을 발현한다. 플라스미드 117은 N-말단으로부터 C-말단으로 SspDnaB(C), Hygro(201-240), NpuDnaE(N)-LZA를 융합함으로써 생성된 마커트론을 발현한다. 플라스미드 118은 LZB-NpuDnaGEP(C)를 Hygro(241-341)로 융합함으로써 생성된 마커트론을 발현한다.

도 12a-12e. Intres 마커는 CRISPR/Cas-매개 녹-인(knock-in) 실험으로부터 이대립유전자 표적화된 세포의 풍부를 허용한다. AAVS1 안전한 하버(harbor) 유전자좌에 대한 상동성 아암을 함유하는 표적화 구축물 쌍은 전장 (FL) 비-분할 또는 분할 Intres 마커를 함유하도록 설계되었고, 항생제 선택을 통해 이대립유전자 표적화된 세포를 풍부하게 하는 능력에 대해 시험되었다. (도 12a) 플라스미드 107 및 108은 AAVS1 유전자좌에서 내인성 PPP1R12C 프로모터에 의해 구동되는 FL 네오마이신 (Neo) 내성 유전자, EF1a 프로모터에 의해 구동되는 FL 히그로마이신 (Hygro) 유전자 및 rtTA Dox-반응성 트랜스활성인자, 뿐만 아니라 dox-유도성 TetO 프로모터로부터 발현된 FL 블라스티시딘 (Blast) 뿐만 아니라 EGFP (플라스미드 107) 및 mScarlet (플라스미드 108)을 함유한다. 플라스미드 106은 Cas9 및 AAVS 유전자좌를 표적화하는 sgRNA를 함유한다. 2A: 자기-절단 2A 펩티드. 플라스미드 106, 107 및 108은 HEK293T 세포로 공동-형질감염되고, 분할되고, dox-함유 히그로마이신, 블라스티시딘 또는 비-선택 배지에서 2주 동안 계대배양되고, 이대립유전자 표적화의 효율을 분석하기 위해 유동 세포계측법에 의해 분석하였다. (도 12b) 플라스미드 109 및 110은 플라스미드 107 및 108과 유사한 구조를 함유하나, FL

Blast 대신에 분할 Blast Intres를 갖는다. (도 12c) 플라스미드 111 및 112는 EF1a-구동된 FL Blast 및 TetO-구동된 FL Hygro, 니트로리덕타제 (NTR), 2A 펩티드에 의해 분리된 형광 단백질 (EGFP 또는 mCherry)을 함유한다. (도 12d) 플라스미드 113 및 114는 플라스미드 111 및 112와 유사하나, FL Hygro 대신에 Hygro Intres를 갖는다. (도 12e) dox-함유 비-선택 배지 (선택: 없음), 블라스티시딘 선택 배지 (Blast) 및 히그로마이신 선택 배지 (Hygro)에서 배양 후 2주에, 플라스미드 106 (Cas9+AAVS-sgRNA)으로 형질감염된 세포 및 표시된 표적화 구축물 쌍의 유동 세포계측법 분석.

발명을 실시하기 위한 구체적인 내용

[0015] <상세한 설명>

[0016] 일부 측면에서, 하나 초과의 트랜스진 (또는 다른 유전적 요소)이 도입된 트랜스제닉 (예를 들어, 다중-트랜스제닉, 예컨대 이중 트랜스제닉 또는 삼중 트랜스제닉) 유기체를 생성하는 방법이 본원에 제공된다. 도 1a에 나타낸 바와 같이, 본 개시내용의 예시적인 방법은 (a) N-말단 인테인 단백질 단편으로부터 상류에 있는 제1 선택가능한 마커 단백질 단편을 코딩하는 벡터 및 관심있는 제1 트랜스진, 및 (b) 제2 선택가능한 마커 단백질 단편으로부터 상류에 있는 C-말단 인테인 단백질 단편을 코딩하는 또 다른 벡터 및 관심있는 제2 (예를 들어, 상이한) 트랜스진을 세포의 집단으로 전달하는 것을 포함한다. 집단의 일부 세포는 단일 벡터 (인테인의 단편, 선택가능한 마커 단백질의 단편, 및 단일 트랜스진만을 보유함)를 수용할 것이나, 집단의 다른 세포는 2종의 벡터 모두 (및 그러므로 인테인 단편 둘 모두, 선택가능한 마커 단백질 단편 둘 모두, 및 관심있는 트랜스진 둘 모두)를 수용할 것이다. 2종의 벡터 모두를 수용하는 세포에서, 번역 후, 인테인 단백질 단편은 인테인 구조로 자발적으로 및 비-공유결합으로 어셈블리하여 (협동적으로 폴딩하여), 제2 선택가능한 마커 단백질 단편으로의 제1 선택가능한 마커 단백질 단편의 연결을 촉매하여, 전장 선택가능한 마커 단백질을 생성하며, 이는 상기 이중 트랜스제닉 세포의 특이적 선택을 가능하게 한다. 예를 들어, 선택가능한 마커 단백질이 항생제 내성 단백질인 경우, 전장 (기능성) 항생제 내성 단백질을 발현하는 이중-트랜스제닉 세포만이 특정 항생제의 존재 하에 선택에서 생존할 것이다. 또 다른 예로서, 선택가능한 마커 단백질이 형광 단백질인 경우, 전장 (기능성) 형광 단백질을 발현하는 이중-트랜스제닉 세포만이 신호-방출 세포만이 선택되도록 검출가능한 신호를 방출할 것이다.

[0017] 본 개시내용의 또 다른 예시적인 방법은 (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터, (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및 (c) (i) 항생제 내성 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터를 세포의 집단으로 전달하는 것을 포함한다. 집단의 일부 세포는 단일 벡터 (인테인의 단편, 선택가능한 마커 단백질의 단편, 및 단일 트랜스진만을 보유함)를 수용할 것이나, 집단의 다른 세포는 2종의 벡터 또는 3종의 벡터 모두 (및 그러므로 인테인 단편 모두, 선택가능한 마커 단백질 단편 모두, 및 관심있는 트랜스진 모두)를 수용할 것이다. 3종의 벡터 모두를 수용하는 세포에서, 번역 후, 인테인 단백질 단편은 인테인 구조로 자발적으로 및 비-공유결합으로 어셈블리하여 (협동적으로 폴딩하여), 선택가능한 마커 단백질의 N-말단 단편의 중심 단편으로의 연결, 및 중심 단편의 선택가능한 마커 단백질의 C-말단 단편으로의 연결을 촉매하여, 전장 선택가능한 마커 단백질을 생성하며, 이는 상기 삼중-트랜스제닉 세포의 특이적 선택을 가능하게 한다. 예를 들어, 선택가능한 마커 단백질이 항생제 내성 단백질인 경우, 전장 (기능성) 항생제 내성 단백질을 발현하는 삼중-트랜스제닉 세포만이 특정 항생제의 존재 하에 선택에서 생존할 것이다. 또 다른 예로서, 선택가능한 마커 단백질이 형광 단백질인 경우, 전장 (기능성) 형광 단백질을 발현하는 삼중-트랜스제닉 세포만이 신호-방출 세포만이 선택되도록 검출가능한 신호를 방출할 것이다.

[0018] 인테인

[0019] 인테인 (개입 단백질)은 단백질 스플라이싱이라고 공지된 고유한 자동-프로세싱 사건을 수행하며, 여기서 이는 2개의 펩티드 결합의 절단을 통해 더 큰 전구체 폴리펩티드로부터 그 자신을 절제하고, 이 과정에서, 새로운 펩티드 결합의 형성을 통해 플랭킹(flanking) 익스테인(extein) (외부 단백질) 서열을 라이게이션한다. 이 재배열은 인테인 유전자가 다른 단백질-코딩 유전자 내에 프레임에 내장된 것으로 발견되기 때문에 번역후 (또는 가능하게는 번역과 공동으로) 발생한다. 더욱이, 인테인-매개 단백질 스플라이싱은 자발적이며; 외부 인자 또는

에너지 공급원이 필요하지 않으며, 오직 인테인 도메인의 폴딩만이 필요하다. 사실상, 전구체 단백질은 3개의 세그먼트, 즉 N-익스테인 (단백질의 N-말단 부분), 이어서 인테인, 이어서 C-익스테인 (단백질의 C-말단 부분)을 함유한다. 스플라이싱 후, 생성된 단백질은 C-익스테인에 연결된 N-익스테인을 함유한다.

[0020] 두 유형의 인테인이 있다: 시스-스플라이싱 인테인은 숙주 단백질에 내장된 단일 폴리펩티드인 반면, 트랜스-스플라이싱 인테인 (분할 인테인이라고 함)은 인테인 조각 및 그의 단백질 카고(cargo)가 회합한 후 단백질 스플라이싱을 매개하는 별도의 폴리펩티드이다 (예를 들어, 문헌 [Paulus, H Annu Rev Biochem 69:447-496 (2000)]; 및 [Saleh L, Perler FB Chem Rec 6:183-193 (2006)] 참조). 분할 인테인은 인테인이 적절히 어셈블리되고 폴딩되는 것을 필요로 하는 일련의 화학적 재배열을 촉매한다. 스플라이싱에서 제1 단계는 N-익스테인 폴리펩티드가 인테인의 제1 잔기의 측쇄로 전이되는 N-S 아실 이동을 수반한다. 그 후, 이는 이 아실 단위가 C-익스테인의 제1 잔기 (이는 세린, 트레오닌 또는 시스테인임)로 전이되어 분지된 중간체를 형성하는 트랜스-(티오)에스테르화 반응으로 이어진다. 프로세스의 끝에서 두 번째 단계에서, 이 분지된 중간체는 인테인의 C-말단 아스파라긴 잔기를 수반하는 아미드 교환 반응에 의해 인테인으로부터 절단된다. 그 후, 이는 두 익스테인 사이에 정상적인 펩티드 결합을 생성하도록 S-N 아실 전이를 수반하는 프로세스의 최종 단계를 설정한다 (Lockless, SW, Muir, TW PNAS 106(27): 10999-11004 (2009)).

[0021] 현재까지, 인테인이 내장된 숙주 유전자의 유형 뿐만 아니라 그 숙주 유전자 내에 통합점에 의해 구별되는 적어도 70종의 상이한 인테인 대립유전자가 있다 (Perler, FB Nucleic Acids Res. 30: 383-384 (2002); Pietrokovski, S Trends Genet. 17: 465-472 (2001)). 확인된 인테인 유전자의 작은 분율 (5% 미만)이 분할 인테인을 코딩한다. 더 흔한 인접 인테인과 달리, 분할 인테인은 각각 하나의 익스테인에 융합된 2개의 별도의 폴리펩티드, N-인테인 및 C-인테인으로서 전사되고 번역된다. 번역시, 인테인 단편은 정준적 인테인 구조로 자발적으로 및 비-공유결합으로 어셈블리하여 (협동적으로 폴딩하여), 단백질 스플라이싱을 트랜스로 수행한다. 시아노박테리아 시네코시스티스(*Synechocystis*) 종 PCC6803 (Ssp) 및 노스톡 푼크티포르메(*Nostoc punctiforme*) PCC73102 (Npu)로부터 특징화된 첫 번째 2개의 분할 인테인은 DNA 폴리머라제 III (DnaE)의 α 서브유닛에 삽입된 것으로 자연적으로 발견되는 오르토로그(ortholog)이다. Npu는 단백질 트랜스-스플라이싱의 현저하게 빠른 속도로 인해 특히 주목할 만하다 ($t_{1/2}$ = 30℃에서 50 s). 이 반감기는 Ssp의 반감기보다 유의하게 짧다 ($t_{1/2}$ = 30℃에서 80 min) (Shah, NH et al. J. Am. Chem. Soc. 135: 5839 (2013)).

[0022] 여기서, 분할 인테인은 선택가능한 마커 단백질, 예컨대 항생제 내성 단백질 또는 형광 단백질의 2개의 단편 (예를 들어, N-말단 단편 및 C-말단 단편)의 연결을 촉매하여 기능성 전장 단백질을 생성하는데 사용된다 (예를 들어, 도 1a 및 1b).

[0023] 분할 인테인은 자연 분할 인테인 또는 조작된 분할 인테인일 수 있다. 자연 분할 인테인은 다양한 상이한 유기체에서 자연적으로 발생한다. 분할 인테인의 가장 큰 공지된 패밀리는 적어도 20종의 시아노박테리아 종의 DnaE 유전자 내에서 발견된다 (Caspi J, et al. Mol. Microbiol. 50: 1569-1577 (2003)). 그러므로, 본 개시내용의 일부 실시양태에서, 자연 분할 인테인은 DnaE 인테인으로부터 선택된다. DnaE 인테인의 비제한적인 예는 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인을 포함한다.

[0024] 일부 실시양태에서, 분할 인테인은 조작된 분할 인테인이다. 조작된 분할 인테인은 인접 인테인으로부터 생성될 수 있거나 (여기서, 인접 인테인은 인공적으로 분할됨), 예를 들어, 효율적인 단백질 정제, 라이게이션, 변형 및 고리화를 촉진하는 변형된 자연 분할 인테인일 수 있다 (예를 들어, Npu_{GEP} 및 Cfa_{GEP}, 문헌 [AJ PNAS 114(32): 8538-8543 (2017)]에 기재된 바와 같음). 분할 인테인을 조작하는 방법은 예를 들어, 본원에 참조로 포함된 문헌 [Aranko, AS et al. Protein Eng Des Sel. 27(8): 263-271 (2014)]에 기재되어 있다. 일부 실시양태에서, 조작된 분할 인테인은 DnaB 인테인으로부터 조작된다 (Wu, H, et al. Biochim Biophys Acta 1387(1-2): 422-432 (1998)). 예를 들어, 조작된 분할 인테인은 SspDnaB S1 인테인일 수 있다. 일부 실시양태에서, 조작된 분할 인테인은 GyrB 인테인으로부터 조작된다. 예를 들어, 조작된 분할 인테인은 SspGyrB S11 인테인일 수 있다.

[0025] 삼중-트랜스제닉이 생성되는 일부 실시양태에서, 예를 들어, 제1 인테인은 제2 인테인과 동일할 수 있다 (예를 들어, 둘 모두 DnaE 인테인). 다른 실시양태에서, 2종의 상이한 인테인이 사용될 수 있다 (예를 들어, DnaE 인테인 및 DnaB 인테인). 일부 실시양태에서, 제1 인테인은 NpuDnaE 인테인이고, 제2 인테인은 NpuDnaE 인테인이다.

[0026] 선택가능한 마커 단백질

[0027] 본 개시내용의 트랜스제닉 (예를 들어, 이중 및/또는 삼중 트랜스제닉) 세포는 전장 선택가능한 마커 단백질의 그의 발현에 기초하여 선택된다. 선택가능한 마커 단백질은 인공 선택에 적합한 형질을 일반적으로 부여한다. 선택가능한 마커 단백질의 예는 항생제 내성 단백질 및 형광 단백질을 포함한다.

[0028] 항생제 내성 유전자는 특정 항생제 또는 항생제의 부류에 대한 내성을 부여하는 단백질을 코딩하는 유전자이다. 진핵생물 세포에서 사용하기 위한 항생제 내성 유전자의 비제한적인 예는 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1 또는 블라스티시딘에 대한 내성을 부여하는 단백질을 코딩하는 유전자를 포함한다. 원핵생물 세포에서 사용하기 위한 항생제 내성 유전자의 비제한적인 예는 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1, 블라스티시딘, 카나마이신, 스펙티노마이신, 스트렙토마이신, 암피실린, 카르베니실린, 블레오마이신, 에리트로마이신, 폴리믹신 D, 테트라사이클린 및 클로람페니콜에 대한 내성을 부여하는 단백질을 코딩하는 유전자를 포함한다.

[0029] 히그로마이신 B는 스트렙토미세스 히그로스코피쿠스(*Streptomyces hygroscopicus*) 박테리아에 의해 생성되는 항생제이다. 이는 단백질 합성을 억제함으로써 박테리아, 진균 및 고등 진핵생물 세포를 사멸시키는 아미노글리코시드이다. 본래 에스케리치아 콜라이(*Escherichia coli*)로부터 유래된 hpt 유전자 (hph 또는 aphIV 유전자라고도 함)에 의해 코딩되는 히그로마이신 포스포트랜스퍼라제 (HPT)는 아미노시클리톨 항생제 히그로마이신 B를 해독한다. 그러므로, 일부 실시양태에서, 본 개시내용의 선택가능한 마커 유전자는 hpt 유전자이다.

[0030] G418 (게네티신®)은 겐타마이신 B1과 구조가 유사한 아미노글리코시드 항생제이다. 이는 미크로모노스포라 로도란게아(*Micromonospora rhodorangea*)에 의해 생성된다. G418은 원핵생물 및 진핵생물 세포 둘 모두에서 신장 단계를 억제함으로써 폴리펩티드 합성을 차단한다. G418에 대한 내성은 아미노글리코시드 3'-포스포트랜스퍼라제, APT 3' II를 코딩하는 Tn5로부터의 neo 유전자에 의해 부여된다. G418은 네오마이신 술페이트의 유사체이며, 네오마이신과 유사한 메커니즘을 갖는다. 그러므로, 일부 실시양태에서, 본 개시내용의 선택가능한 마커 유전자는 neo 유전자이다.

[0031] 퓨로마이신은 스트렙토미세스 알보니게르(*Streptomyces alboniger*)로부터 유래된 아미노뉴클레오시드 항생제이며, 리보솜에서 일어나는 번역 동안 조기 쇄 종결을 야기한다. 퓨로마이신은 원핵생물 또는 진핵생물에 대해 선택적이다. 퓨로마이신에 대한 내성은 퓨로마이신 N-아세틸-트랜스퍼라제 (pac) 유전자의 발현을 통해 부여된다. 그러므로, 일부 실시양태에서, 본 개시내용의 선택가능한 마커 유전자는 pac 유전자이다.

[0032] 플레오마이신 D1 (예를 들어, 제오신®)은 글리코펩티드 항생제이며, 항생제의 블레오마이신 패밀리에 속하는 스트렙토미세스 베르티실루스(*Streptomyces verticillus*)로부터의 플레오마이신 중 하나이다. 이는 대부분의 박테리아, 사상 진균, 효모, 식물 및 동물 세포에 대해 효과적인 광범위-스펙트럼 항생제이다. 이는 DNA에 삽입함으로써 세포 사멸을 야기하고 DNA의 이중 가닥 파단을 유도한다. 플레오마이신 D1에 대한 내성은 스트렙토알로테이쿠스 힌두스타누스(*Streptoalloteichus hindustanus*)로부터 처음 단리된 Sh ble 유전자의 생성물에 의해 부여된다. 그러므로, 일부 실시양태에서, 본 개시내용의 선택가능한 마커 유전자는 Sh ble 유전자이다.

[0033] 블라스티시딘 S는 스트렙토미세스 그리세오크로모게네스(*Streptomyces griseochromogenes*)에 의해 생성되는 항생제이다. 블라스티시딘은 번역의 종결 단계 및 리보솜에 의한 펩티드 결합 형성 (더 적은 정도로)을 억제함으로써 진핵생물 및 원핵생물 세포 둘 모두의 성장을 방지한다. 블라스티시딘에 대한 내성은 적어도 3종의 상이한 유전자에 의해 부여된다: 스트렙토베르티실룸(*Streptoverticillum*) spp.로부터의 bls (아세틸트랜스퍼라제); 바실러스 세레우스(Bacillus cereus)로부터의 bsr (블라스티시딘-S 데아미나제) (다른 bsr 유전자도 공지되어 있음); 및 아스페르길루스 테레우스(*Aspergillus terreus*)로부터의 bsd (또 다른 데아미나제). 그러므로, 일부 실시양태에서, 본 개시내용의 선택가능한 마커 유전자는 bls 유전자, bsr 유전자 또는 bsd 유전자이다.

[0034] 본원에 제공된 바와 같이 사용될 수 있는 형광 단백질의 비제한적인 예는 TagCFP, mTagCFP2, Czurite, ECFP2, mKalama1, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3C, mTurquoise, mTurquoise2, 모노머 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, 모노머 Czami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, 모노머 Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mScarlet, mTangerine, tdTomato, TagRFP, TagRFP-T, mCpple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, TagRFP657, IFP1.4 및 iRFP를 포함한다.

[0035] 일부 실시양태에서, 전장 선택가능한 마커 유전자는 동일한 세포에서 2개의 선택가능한 마커 유전자 단편을 연결함으로써 생성된다. 일부 실시양태에서, 임의의 전장 단백질에 관하여, 단편 중 하나는 N-말단 단편 (N-익스

테인)인 반면, 다른 단편은 C-말단 단편 (C-익스테인)이다. 그러므로, 일부 실시양태에서, 제1 항생제 내성 단백질 단편은 N-말단 항생제 내성 단백질 단편이고, 제2 항생제 내성 단백질 단편은 C-말단 항생제 내성 단백질 단편이다. 다른 실시양태에서, 제1 형광 단백질 단편은 N-말단 형광 단백질 단편이고, 제2 형광 단백질 단편은 C-말단 형광 단백질 단편이다.

[0036] 다른 실시양태에서, 전장 선택가능한 마커 유전자는 동일한 세포에서 3개 이상의 선택가능한 마커 유전자 단편을 연결함으로써 생성된다. 일부 실시양태에서, 임의의 전장 단백질에 관하여, 단편 중 하나는 N-말단 단편이고, 단편 중 하나 이상 (예를 들어, 1, 2 또는 3개)은 중심 단편이고, 단편 중 하나는 C-말단 단편이다.

[0037] N-말단 단편은 전장 단백질의 유리 아민 기 (-NH2)를 포함하는 임의의 단백질 단편일 수 있다. C-말단 단편은 유리 카르복실 기 (-COOH)를 포함하는 임의의 단백질 단편일 수 있다. 중심 단편은 전장 단백질의 N-말단 단편 및 C-말단 단편 사이에 위치된 임의의 단백질 단편일 수 있다.

[0038] 예를 들어, 유전자 코딩 히그로마이신 (341-아미노산 단백질)의 아미노산 1-89는 N-말단 단백질 단편으로서 지칭될 수 있는 반면, 아미노산 90-341은 C-말단 단편으로서 지칭될 수 있다. 유사하게, 도 5에 관하여, 히그로마이신을 코딩하는 유전자의 아미노산 1-200은 N-말단 단백질 단편으로서 지칭될 수 있는 반면, 아미노산 201-341은 C-말단 단편으로서 지칭될 수 있다. 도 6은 아미노산 1-53, 1-240, 또는 1-292가 각각의 C-말단 단편으로서 아미노산 54-341, 241-341, 또는 293-341을 함유하는 전장 히그로마이신의 N-말단 단백질 단편으로 간주되는 추가 예를 나타낸다.

[0039] 또 다른 예로서, 유전자 코딩 히그로마이신 (341-아미노산 단백질)의 아미노산 1-52는 N-말단 단백질 단편으로서 지칭될 수 있고, 아미노산 53-89는 중심 단백질 단편으로서 지칭될 수 있고, 아미노산 90-341은 C-말단 단편으로서 지칭될 수 있다. 유사하게, 히그로마이신을 코딩하는 유전자의 아미노산 1-89는 N-말단 단백질 단편으로서 지칭될 수 있고, 아미노산 90-240은 중심 단편으로서 지칭될 수 있고, 아미노산 241-341은 C-말단 단편으로서 지칭될 수 있다.

[0040] 관심있는 트랜스진 및 다른 분자

[0041] 일부 실시양태에서, 본 개시내용의 방법 및 조성물은 다중-트랜스제닉 (예를 들어, 이중 및/또는 삼중 트랜스제닉) 세포 및/또는 유기체를 생성하는데 사용된다. 그러므로, 일부 실시양태에서, 방법은 제1 분자 (관심있는 제1 분자)를 코딩하는 하나의 벡터 및 제2 분자 (관심있는 제2 분자)를 코딩하는 또 다른 벡터를 사용한다. 일부 실시양태에서, 방법은 관심있는 제3 분자를 코딩하는 또 다른 벡터를 사용한다. 추가 벡터 (예를 들어, 선택가능한 마커 단백질의 추가 중심 단편을 코딩함)는 관심있는 추가 분자를 코딩할 수 있다. 관심있는 분자는 예를 들어, 폴리펩티드 (예를 들어, 단백질 및 펩티드) 또는 폴리뉴클레오티드 (예를 들어, 핵산, 예컨대 DNA 또는 RNA)일 수 있다.

[0042] 일부 실시양태에서, 제1 분자 (예를 들어, 제1 벡터 상에 위치됨)는 단백질이다. 일부 실시양태에서, 제2 분자 (예를 들어, 제2 벡터 상에 위치됨)는 단백질이다. 일부 실시양태에서, 제3 분자 (예를 들어, 제3 벡터 상에 위치됨)는 단백질이다. 관심있는 단백질의 예는 효소, 시토카인, 전사 인자, 호르몬, 성장 인자, 혈액 인자, 항원 및 항체를 포함하나, 이에 제한되지는 않는다.

[0043] 일부 실시양태에서, 제1 분자는 펩티드이다. 일부 실시양태에서, 제2 분자는 펩티드이다. 일부 실시양태에서, 제3 분자는 펩티드이다.

[0044] 일부 실시양태에서, 제1 분자는 메신저 RNA (mRNA)이다. 일부 실시양태에서, 제2 분자는 mRNA이다. 일부 실시양태에서, 제3 분자는 mRNA이다. 일부 실시양태에서, mRNA는 백신 또는 다른 항원성 분자를 코딩한다.

[0045] 일부 실시양태에서, 제1 분자는 비-코딩 RNA (단백질을 코딩하지 않는 RNA)이다. 일부 실시양태에서, 제2 분자는 비-코딩 RNA이다. 일부 실시양태에서, 제3 분자는 비-코딩 RNA이다. 비-코딩 RNA의 예는 RNA 간섭 분자, 예컨대 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)를 포함하나, 이에 제한되지는 않는다.

[0046] 벡터

[0047] 본 개시내용의 방법은 적어도 2종 또는 적어도 3종의 상이한 벡터의 사용을 포함한다. 벡터는 외인성 (외래) 유전 물질을 세포로 운반하는 비히클로서 사용될 수 있는 임의의 핵산이다. 일부 실시양태에서, 벡터는 삽입물 (예를 들어, 트랜스진) 및 벡터의 백본으로서 작용하는 더 큰 서열을 포함하는 DNA 서열이다. 벡터의 비제한적인 예는 플라스미드, 바이러스/바이러스 벡터, 코스미드, 및 인공 염색체를 포함하며, 이들 중 임의의 것이 본

원에 제공된 바와 같이 사용될 수 있다. 일부 실시양태에서, 벡터는 바이러스 벡터, 예컨대 바이러스 입자이다. 일부 실시양태에서, 벡터는 RNA-기반 벡터, 예컨대 자기-복제 RNA 벡터이다. 일부 실시양태에서, 제1 벡터는 플라스미드이고/거나, 제2 벡터는 플라스미드이고/거나, 제3 벡터는 플라스미드이다. 본원에 제공된 바와 같은 벡터는 인테인의 단편 및 선택가능한 마커 단백질의 단편을 코딩하는 핵산에 작동가능하게 연결된 프로모터를 포함한다. 일부 실시양태에서, 벡터는 또한 관심있는 분자를 코딩하는 핵산, 예컨대 트랜스진에 작동가능하게 연결된 프로모터를 포함한다.

[0048] 일부 실시양태에서, 하나의 벡터 (예를 들어, 제1 벡터)는 N-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는 제1 선택가능한 마커 단백질 단편을 코딩하는 뉴클레오티드 서열을 포함하는 반면, 다른 하나의 벡터 (예를 들어, 제2 벡터)는 제2 항생제 내성 단백질 단편으로부터 상류에 있는 C-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열을 포함한다 (예를 들어, 도 1a 참조). 이 구성은 제1 선택가능한 마커 단백질 단편을 코딩하는 뉴클레오티드 서열로부터 하류에 있는 N-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열을 포함하는 하나의 벡터 (예를 들어, 제1 벡터), 및 C-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열로부터 하류에 있는 제2 항생제 내성 단백질 단편을 포함하는 다른 하나의 벡터 (예를 들어, 제2 벡터)와 동등하다. 용어 "상류" 및 "하류"는 핵산에서의 상대 위치를 지칭한다. 각각의 핵산은 데옥시리보스 (또는 리보스) 고리 상의 탄소 위치로 명명되는 5' 말단 및 3' 말단을 갖는다. 예를 들어, 이중-가닥 DNA를 고려할 때, 상류는 코딩 가닥의 5' 말단을 향하고, 하류는 3' 말단을 향한다.

[0049] 일부 실시양태에서, (a) 제1 벡터는 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하고, (b) 제2 벡터는 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하고, (c) 제3 벡터는 항생제 내성 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함한다. 이 구성은 a) 항생제 내성 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 하류에 있는, 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터, (b) 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 하류에 있는, 항생제 내성 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 하류에 있는, 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및 (c) 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 하류에 있는, 항생제 내성 단백질의 C-말단 단편을 포함하는 제3 벡터와 동등하다.

[0050] 세포

[0051] 본 개시내용의 방법은 본원에 기재된 벡터 (예를 들어, 제1 및 제2 벡터)를 숙주 세포로 도입함으로써 트랜스제닉 세포 및 유기체를 생성하는데 사용될 수 있다. 벡터가 도입된 세포는 진핵생물 또는 원핵생물일 수 있다. 일부 실시양태에서, 세포는 진핵생물이다. 본원에 제공된 바와 같이 사용하기 위한 진핵생물 세포의 예는 포유동물 세포, 식물 세포 (예를 들어, 작물 세포), 곤충 세포 (예를 들어, 드로소필라(*Drosophila*)) 및 진균 세포 (예를 들어, 사카로미세스(*Saccharomyces*))를 포함한다. 포유동물 세포는 예를 들어, 인간 세포 (줄기 세포 또는 확립된 세포주로부터의 세포), 영장류 세포, 말 세포, 소 세포, 돼지 세포, 개 세포, 고양이 세포, 또는 설치류 세포 (예를 들어, 마우스 또는 래트)일 수 있다. 본원에 제공된 바와 같이 사용하기 위한 포유동물 세포의 예는 차이니즈 햄스터 난소 (CHO) 세포, 인간 배아 신장 (HEK) 293 세포, HeLa 세포 및 NS0 세포를 포함하나, 이에 제한되지는 않는다. 일부 실시양태에서, 세포는 원핵생물이다. 본원에 제공된 바와 같이 사용하기 위한 원핵생물 세포의 예는 박테리아 세포를 포함한다. 박테리아 세포는 예를 들어, 에스케리치아(*Escherichia*) spp. (예를 들어, 에스케리치아 콜라이), 스트렙토코쿠스(*Streptococcus*) spp. (예를 들어, 스트렙토코쿠스 피오게네스(*Streptococcus pyogenes*), 스트렙토코쿠스 비리단스(*Streptococcus viridans*), 스트렙토코쿠스 뉴모니아에(*Streptococcus pneumoniae*)), 네이쎄리아(*Neisseria*) spp. (예를 들어, 네이쎄리아 기비로에아(*Neisseria gibirrhoea*), 네이쎄리아 메닌기티디스(*Neisseria meningitidis*)), 코리네박테리움(*Corynebacterium*) spp. (예를 들어, 코리네박테리움 디프테리아에(*Corynebacterium diphtheriae*)), 바실리스(*Bacillis*) spp. (예를 들어, 바실리스 안트라시스(*Bacillis anthracis*), 바실리스 서브틸리스(*Bacillis subtilis*)), 락토바실러스(*Lactobacillus*) spp., 클로스트리디움(*Clostridium*) spp. (예를 들어, 클로스트리디움 테타니(*Clostridium tetani*), 클로스트리디움 페르프린겐스(*Clostridium perfringens*), 클로스트리디움 노비이(*Clostridium novyii*)), 미코박테리움(*Mycobacterium*) spp. (예를 들어, 미코박테리움 투베르쿨로시스(*Mycobacterium tuberculosis*)), 시겔라(*Shigella*) spp. (예를 들어, 시겔라 플렉스네리(*Shigella flexneri*), 시겔라 디센테리아에(*Shigella dysenteriae*)), 살모넬라(*Salmonella*) spp. (예를 들어, 살모넬라 티피

(*Salmonella typhi*), 살모넬라 엔테리티디스(*Salmonella enteritidis*)), 클렙시엘라(*Klebsiella*) spp. (예를 들어, 클렙시엘라 뉴모니아에(*Klebsiella pneumoniae*)), 예르시니아(*Yersinia*) spp. (예를 들어, 예르시니아 페스티스(*Yersinia pestis*)), 세라티아(*Serratia*) spp. (예를 들어, 세라티아 마르세센스(*Serratia marcescens*)), 슈도모나스(*Pseudomonas*) spp. (예를 들어, 슈도모나스 아에루기노사(*Pseudomonas aeruginosa*), 슈도모나스 말레이(*Pseudomonas mallei*)), 에이케넬라(*Eikenella*) spp. (예를 들어, 에이케넬라 코로덴스(*Eikenella corrodens*)), 하에모필루스(*Haemophilus*) spp. (예를 들어, 하에모필루스 인플루엔자(*Haemophilus influenza*), 하에모필루스 두크레이이(*Haemophilus ducreyi*), 하에모필루스 아에깁티우스(*Haemophilus aegyptius*)), 비브리오(*Vibrio*) spp. (예를 들어, 비브리오 콜레라(*Vibrio cholera*), 비브리오 나트리에겐스(*Vibrio natriegens*)), 레기오넬라(*Legionella*) spp. (예를 들어, 레기오넬라 미크다데이(*Legionella micdadei*), 레기오넬라 보제마니(*Legionella bozemani*)), 브루셀라(*Brucella*) spp. (예를 들어, 브루셀라 아보르투스(*Brucella abortus*)), 미코플라스마(*Mycoplasma*) spp. (예를 들어, 미코플라스마 뉴모니아에(*Mycoplasma pneumoniae*)) 또는 스트렙토미세스(*Streptomyces*) spp. (예를 들어 스트렙토미세스 코엘리콜로르(*Streptomyces coelicolor*), 스트렙토미세스 리비단스(*Streptomyces lividans*), 스트렙토미세스 알부스(*Streptomyces albus*))일 수 있다.

[0052] 전달 및 선택 방법

[0053] 일부 실시양태에서, 본 개시내용의 방법은 세포를 포함하는 조성물로 벡터를 전달하고, 진핵생물 세포를 생성하기 위해 세포로의 핵산 (예를 들어, 제1, 제2 및 제3 벡터)의 도입을 허용하고 세포에서 핵산 발현을 허용하는 조건 하에 조성물을 유지하는 것을 포함한다. 세포로의 핵산 (예를 들어, 벡터)의 도입에 필요한 조건은 널리 공지되어 있다. 이들 조건은 예를 들어, (원핵생물 세포의) 형질전환 조건, (진핵생물 세포의) 형질감염 조건, (바이러스/바이러스 벡터를 통한) 형질도입 조건, 및 전기천공 조건을 포함하며, 이들 중 임의의 것이 본원에 제공된 바와 같이 사용될 수 있다. 그러므로, 일부 실시양태에서, 본 개시내용의 방법은 진핵생물 (예를 들어 포유동물) 세포를 형질감염시키는 것을 포함하는 반면, 다른 실시양태에서, 방법은 원핵생물 (예를 들어, 박테리아) 세포를 형질전환시키는 것을 포함한다.

[0054] 트랜스제닉, 예를 들어, 다중-트랜스제닉 세포, 예컨대 이중, 삼중, 및/또는 사중 트랜스제닉 세포의 선택은 사용되는 선택가능한 마커의 유형에 좌우된다. 예를 들어, 선택가능한 마커 단백질이 항생제 내성 단백질인 경우, 선택 단계는 세포를 특정 항생제에 노출시키고, 생존하는 세포만을 선택하는 것을 포함할 수 있다. 선택가능한 마커 단백질이 형광 단백질인 경우, 선택 단계는 단순히 세포를 현미경 하에서 관찰하고, 형광을 내는 세포를 선택하는 것을 포함할 수 있거나, 선택 단계는 다른 형광 선택 방법, 예컨대 형광-활성화 세포 분류법 (FACS) 분류법을 포함할 수 있다.

[0055] 일부 실시양태에서, 세포는 본원에 기재된 바와 같은 핵산을 보유하는 바이러스 벡터 (예를 들어, 바이러스)로 형질도입된다. 일부 실시양태에서, 형질도입 (또는 다른 형질감염 방법) 전에, 세포를 예를 들어, 웰 당 $1x10^4$ 내지 $1x10^6$개의 밀도로 웰 플레이트 (예를 들어, 12-웰 플레이트) 상에 씨딩한다. 일부 실시양태에서, 100 μL 내지 500 μL, 예를 들어, 100, 150, 200, 250, 300, 350, 400, 450, 또는 500 μL의 각각의 바이러스 벡터를 각각의 웰에 첨가한다.

[0056] 키트

[0057] 본 개시내용은 또한 예를 들어, 트랜스제닉 세포 및/또는 유기체를 생성하고 스크리닝하는데 사용될 수 있는 키트를 제공한다. 키트는 본원에 기재된 바와 같은 임의의 둘 이상의 구성성분을 포함할 수 있다. 예를 들어, 키트는 (a) N-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 선택가능한 마커 단백질 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터; 및 (b) 제2 선택가능한 마커 단백질 단편으로부터 상류에 있는, C-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터를 포함할 수 있으며, 여기서 N-말단 인테인 단백질 단편 및 C-말단 인테인 단백질 단편은 제2 선택가능한 마커 단백질 단편으로의 제1 선택가능한 마커 단백질 단편의 연결을 촉매하여 전장 항생제 내성 단백질을 생성한다.

[0058] 일부 실시양태에서, 키트는 본원에 기재된 바와 같은 임의의 둘 이상의 구성성분을 포함한다. 예를 들어, 키트는 (a) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터, (b) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및 (c) 항생제 내성

단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터를 포함할 수 있으며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 중심 단편으로의 항생제 내성 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 C-말단 단편으로의 항생제 내성 단백질의 중심 단편의 연결을 촉매하여 전장 항생제 내성 단백질을 생성한다.

[0059] 일부 실시양태에서, 키트는 다음 구성성분: 완충제, 염, 클로닝 효소 (예를 들어, LR 클로나제), 감응성 세포 (예를 들어, 감응성 박테리아 세포), 형질감염 시약, 항생제, 및/또는 본원에 기재된 방법의 수행에 대한 지침서 중 임의의 하나 이상을 추가로 포함한다.

[0060] <추가 실시양태>

[0061] 본 개시내용의 추가 실시양태는 하기 번호의 단락에 포함된다:

[0062] 1. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터; 및

[0063] (b) (i) 항생제 내성 단백질의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터

[0064] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 N-말단 단편 및 C-말단 단편의 연결을 촉매하여 전장 항생제 내성 단백질을 생성하는 것인 방법.

[0065] 2. 단락 1에 있어서, 트랜스제닉 진핵생물 세포를 생성하기 위해 진핵생물 세포로의 제1 및 제2 벡터의 도입을 허용하는 조건 하에 진핵생물 세포를 유지하는 것을 추가로 포함하는 방법.

[0066] 3. 단락 2에 있어서, 전장 항생제 내성 단백질을 포함하는 트랜스제닉 진핵생물 세포를 선택하는 것을 추가로 포함하는 방법.

[0067] 4. 단락 1 내지 3 중 어느 한 단락에 있어서, 진핵생물 세포가 포유동물 세포인 방법.

[0068] 5. 단락 1 내지 4 중 어느 한 단락에 있어서, 항생제 내성 단백질이 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1 또는 블라스티시딘에 대한 내성을 부여하는 것인 방법.

[0069] 6. 단락 1 내지 5 중 어느 한 단락에 있어서, 인테인이 분할 인테인인 방법.

[0070] 7. 단락 6에 있어서, 분할 인테인이 자연 분할 인테인인 방법.

[0071] 8. 단락 7에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 방법.

[0072] 9. 단락 8에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 방법.

[0073] 10. 단락 6에 있어서, 분할 인테인이 조작된 분할 인테인인 방법.

[0074] 11. 단락 10에 있어서, 조작된 분할 인테인이 DnaB 인테인으로부터 조작된 것인 방법.

[0075] 12. 단락 11에 있어서, 조작된 분할 인테인이 SspDnaB S1 인테인인 방법.

[0076] 13. 단락 12에 있어서, 조작된 분할 인테인이 GyrB 인테인으로부터 조작된 것인 방법.

[0077] 14. 단락 13에 있어서, 조작된 분할 인테인이 SspGyrB S11 인테인인 방법.

[0078] 15. 단락 1 내지 14 중 어느 한 단락에 있어서, 제1 및/또는 제2 분자가 단백질인 방법.

[0079] 16. 단락 1 내지 15 중 어느 한 단락에 있어서, 제1 및/또는 제2 분자가 비-코딩 리보핵산 (RNA)인 방법.

[0080] 17. 단락 16에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 방법.

[0081] 18. 단락 1 내지 17 중 어느 한 단락에 있어서, 제1 및/또는 제2 벡터가 플라스미드 벡터 또는 바이러스 벡터인 방법.

[0082] 19. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, hygB 유전자의 N-말단 단

편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0083] (b) (ii) hygB 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0084] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 히그로마이신 B 포스포트랜스퍼라제를 생성하는 것인 방법.

[0085] 20. 단락 19에 있어서, 제2 hygB 유전자 단편에 의해 코딩된 단백질 단편의 첫 번째 아미노산이 시스테인인 방법.

[0086] 21. 단락 23에 있어서,

[0087] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-89에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 90-341에 의해 확인된 아미노산 서열을 포함하거나;

[0088] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-200에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 201-341에 의해 확인된 아미노산 서열을 포함하거나;

[0089] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-53에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 54-341에 의해 확인된 아미노산 서열을 포함하거나;

[0090] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-240에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 241-341에 의해 확인된 아미노산 서열을 포함하거나;

[0091] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-292에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 293-341에 의해 확인된 아미노산 서열을 포함하는 것인 방법.

[0092] 22. 단락 23 내지 21 중 어느 한 단락에 있어서,

[0093] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0094] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0095] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 방법.

[0096] 23. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, bsr 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0097] (b) (ii) bsr 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0098] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 bsr 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 bsr 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 블라스티시딘-S 데아미나제를 생성하는 것인 방법.

[0099] 24. 단락 23에 있어서, bsr 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 4의 아미노산 1-102에 의해 확인된 아미노산 서열을 포함하고, bsr 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 4의 아미노산 103-140에 의해 확인된 아미노산 서열을 포함하는 것인 방법.

[0100] 25. 단락 22 또는 23에 있어서,

[0101] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해

확인되거나;

[0102] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0103] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 방법.

[0104] 26. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, pac 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0105] (b) (ii) pac 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0106] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 pac 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 pac 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 퓨로마이신 N-아세틸-트랜스퍼라제를 생성하는 것인 방법.

[0107] 27. 단락 26에 있어서,

[0108] pac 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 1-63에 의해 확인된 아미노산 서열을 포함하고, pac 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 64-199에 의해 확인된 아미노산 서열을 포함하거나;

[0109] pac 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 1-119에 의해 확인된 아미노산 서열을 포함하고, pac 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 120-199에 의해 확인된 아미노산 서열을 포함하거나;

[0110] pac 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 1-100에 의해 확인된 아미노산 서열을 포함하고, pac 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 101-199에 의해 확인된 아미노산 서열을 포함하는 것인 방법.

[0111] 28. 단락 26 또는 27에 있어서,

[0112] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0113] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0114] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 방법.

[0115] 29. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, neo 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0116] (b) (ii) neo 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0117] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 neo 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 neo 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 아미노글리코시드 3'-포스포트랜스퍼라제를 생성하는 것인 방법.

[0118] 30. 단락 29에 있어서,

[0119] neo 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 3의 아미노산 1-133에 의해 확인된 아미노산 서열을 포함하고, neo 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 3의 아미노산 134-267에 의해 확인된 아미노산 서열을 포함하거나; 또는

[0120] neo 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 3의 아미노산 1-194에 의해 확인된 아미노산 서열을 포함하고, neo 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 3의 아미노산 195-267에 의해 확인된 아미노산 서열을 포함하는 것인 방법.

[0121] 31. 단락 29 또는 30에 있어서,

[0122] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0123] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0124] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 방법.

[0125] 32. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 형광 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터; 및

[0126] (b) (ii) 형광 단백질의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터

[0127] 를 진핵생물 세포를 포함하는 조성물로 전달하는 것을 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 형광 단백질의 C-말단 단편으로의 형광 단백질의 N-말단 단편의 연결을 촉매하여 전장 형광 단백질을 생성하는 것인 방법.

[0128] 33. 단락 51에 있어서, 트랜스제닉 진핵생물 세포를 생성하기 위해 진핵생물 세포로의 제1 및 제2 벡터의 도입을 허용하는 조건 하에 진핵생물 세포를 유지하는 것을 추가로 포함하는 방법.

[0129] 34. 단락 33에 있어서, 전장 형광 단백질을 포함하는 트랜스제닉 진핵생물 세포를 선택하는 것을 추가로 포함하는 방법.

[0130] 35. 단락 32 내지 34 중 어느 한 단락에 있어서, 진핵생물 세포가 포유동물 세포인 방법.

[0131] 36. 단락 32 내지 35 중 어느 한 단락에 있어서, 형광 단백질이 TagCFP, mTagCFP2, Czurite, ECFP2, mKalama1, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3C, mTurquoise, mTurquoise2, 모노머 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, 모노머 Czami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, 모노머 Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mCpple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, TagRFP657, IFP1.4 및 iRFP로부터 선택된 것인 방법.

[0132] 37. 단락 32 내지 36 중 어느 한 단락에 있어서, 인테인이 분할 인테인인 방법.

[0133] 38. 단락 37에 있어서, 분할 인테인이 자연 분할 인테인인 방법.

[0134] 39. 단락 38에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 방법.

[0135] 40. 단락 39에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 방법.

[0136] 41. 단락 40에 있어서, 분할 인테인이 조작된 분할 인테인인 방법.

[0137] 42. 단락 41에 있어서, 조작된 분할 인테인이 DnaB 인테인으로부터 조작된 것인 방법.

[0138] 43. 단락 42에 있어서, 조작된 분할 인테인이 SspDnaB S1 인테인인 방법.

[0139] 44. 단락 42에 있어서, 조작된 분할 인테인이 GyrB 인테인으로부터 조작된 것인 방법.

[0140] 45. 단락 44에 있어서, 조작된 분할 인테인이 SspGyrB S11 인테인인 방법.

[0141] 46. 단락 32 내지 45 중 어느 한 단락에 있어서, 제1 및/또는 제2 분자가 단백질인 방법.

[0142] 47. 단락 32 내지 46 중 어느 한 단락에 있어서, 제1 및/또는 제2 분자가 비-코딩 리보핵산 (RNA)인 방법.

[0143] 48. 단락 47에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 방법.

[0144] 49. 단락 32 내지 48 중 어느 한 단락에 있어서, 제1 및/또는 제2 벡터가 플라스미드 벡터 또는 바이러스 벡터

인 방법.

[0145] 50. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, egfp 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0146] (b) (ii) egfp 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0147] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 egfp 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 egfp 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 EGFP 단백질을 생성하는 것인 방법.

[0148] 51. 단락 50에 있어서, egfp 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 5의 아미노산 1-175에 의해 확인된 아미노산 서열을 포함하고, egfp 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 5의 아미노산 175-239에 의해 확인된 아미노산 서열을 포함하는 것인 방법.

[0149] 52. 단락 50 또는 51에 있어서,

[0150] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0151] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0152] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 방법.

[0153] 53. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, mScarlet 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0154] (b) (ii) mScarlet 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0155] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 mScarlet 단백질을 생성하는 것인 방법.

[0156] 54. 단락 53에 있어서,

[0157] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-46에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 47-232에 의해 확인된 아미노산 서열을 포함하거나;

[0158] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-48에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 49-232에 의해 확인된 아미노산 서열을 포함하거나;

[0159] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-51에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 52-232에 의해 확인된 아미노산 서열을 포함하거나;

[0160] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-75에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 76-232에 의해 확인된 아미노산 서열을 포함하거나;

[0161] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-122에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 123-232에 의해 확인된 아미노산 서열을 포함하거나;

[0162] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-140에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아

미노산 141-232에 의해 확인된 아미노산 서열을 포함하거나;

[0163] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-163에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 164-232에 의해 확인된 아미노산 서열을 포함하는 것인 방법.

[0164] 55. 단락 53 또는 54에 있어서,

[0165] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0166] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0167] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 방법.

[0168] 56. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터; 및

[0169] (b) (i) 항생제 내성 단백질의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터

[0170] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 N-말단 단편 및 C-말단 단편의 연결을 촉매하여 전장 항생제 내성 단백질을 생성하는 것인 진핵생물 세포.

[0171] 57. 단락 56에 있어서, 진핵생물 세포가 포유동물 세포인 세포.

[0172] 58. 단락 56 또는 57에 있어서, 항생제 내성 단백질이 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1 또는 블라스티시딘에 대한 내성을 부여하는 것인 세포.

[0173] 59. 단락 56 내지 58 중 어느 한 단락에 있어서, 인테인이 분할 인테인인 세포.

[0174] 60. 단락 59에 있어서, 분할 인테인이 자연 분할 인테인인 세포.

[0175] 61. 단락 60에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 세포.

[0176] 62. 단락 61에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 세포.

[0177] 63. 단락 59에 있어서, 분할 인테인이 조작된 분할 인테인인 세포.

[0178] 64. 단락 63에 있어서, 조작된 분할 인테인이 DnaB 인테인으로부터 조작된 것인 세포.

[0179] 65. 단락 64에 있어서, 조작된 분할 인테인이 SspDnaB S1 인테인인 세포.

[0180] 66. 단락 65에 있어서, 조작된 분할 인테인이 GyrB 인테인으로부터 조작된 것인 세포.

[0181] 67. 단락 66에 있어서, 조작된 분할 인테인이 SspGyrB S11 인테인인 세포.

[0182] 68. 단락 56 내지 67 중 어느 한 단락에 있어서, 제1 및/또는 제2 분자가 단백질인 세포.

[0183] 69. 단락 56 내지 68 중 어느 한 단락에 있어서, 제1 및/또는 제2 분자가 비-코딩 리보핵산 (RNA)인 세포.

[0184] 70. 단락 69에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 세포.

[0185] 71. 단락 56 내지 70 중 어느 한 단락에 있어서, 제1 및/또는 제2 벡터가 플라스미드 벡터 또는 바이러스 벡터인 세포.

[0186] 72. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, hygB 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0187] (b) (ii) hygB 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서

열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0188] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 히그로마이신 B 포스포트랜스퍼라제를 생성하는 것인 세포.

[0189] 73. 단락 72에 있어서, 제2 hygB 유전자 단편에 의해 코딩된 단백질 단편의 첫 번째 아미노산이 시스테인인 세포.

[0190] 74. 단락 73에 있어서,

[0191] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-89에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 90-341에 의해 확인된 아미노산 서열을 포함하거나;

[0192] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-200에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 201-341에 의해 확인된 아미노산 서열을 포함하거나;

[0193] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-53에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 54-341에 의해 확인된 아미노산 서열을 포함하거나;

[0194] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-240에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 241-341에 의해 확인된 아미노산 서열을 포함하거나;

[0195] hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 1-292에 의해 확인된 아미노산 서열을 포함하고, hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 1의 아미노산 293-341에 의해 확인된 아미노산 서열을 포함하는 것인 세포.

[0196] 75. 단락 72 내지 74 중 어느 한 단락에 있어서,

[0197] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0198] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0199] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 세포.

[0200] 76. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, bsr 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0201] (b) (ii) bsr 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0202] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 bsr 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 bsr 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 블라스티시딘-S 데아미나제를 생성하는 것인 진핵생물 세포.

[0203] 77. 단락 76에 있어서, bsr 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 4의 아미노산 1-102에 의해 확인된 아미노산 서열을 포함하고, bsr 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 4의 아미노산 103-140에 의해 확인된 아미노산 서열을 포함하는 것인 세포.

[0204] 78. 단락 76 또는 77에 있어서,

[0205] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0206] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확

인되거나; 또는

[0207] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 세포.

[0208] 79. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, pac 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0209] (b) (ii) pac 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0210] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 pac 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 pac 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 퓨로마이신 N-아세틸-트랜스퍼라제를 생성하는 것인 진핵생물 세포.

[0211] 80. 단락 79에 있어서,

[0212] pac 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 1-63에 의해 확인된 아미노산 서열을 포함하고, pac 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 64-199에 의해 확인된 아미노산 서열을 포함하거나;

[0213] pac 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 1-119에 의해 확인된 아미노산 서열을 포함하고, pac 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 120-199에 의해 확인된 아미노산 서열을 포함하거나;

[0214] pac 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 1-100에 의해 확인된 아미노산 서열을 포함하고, pac 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 2의 아미노산 101-199에 의해 확인된 아미노산 서열을 포함하는 것인 세포.

[0215] 81. 단락 79 또는 80에 있어서,

[0216] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0217] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0218] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 세포.

[0219] 82. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, neo 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0220] (b) (ii) neo 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0221] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 neo 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 neo 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 아미노글리코시드 3'-포스포트랜스퍼라제를 생성하는 것인 진핵생물 세포.

[0222] 83. 단락 82에 있어서,

[0223] neo 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 3의 아미노산 1-133에 의해 확인된 아미노산 서열을 포함하고, neo 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 3의 아미노산 134-267에 의해 확인된 아미노산 서열을 포함하거나; 또는

[0224] neo 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 3의 아미노산 1-194에 의해 확인된 아미노산 서열을 포함하고, neo 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 3의 아미노산 195-267에 의해 확인된 아미노산 서열을 포함하는 것인 세포.

[0225] 84. 단락 82 또는 83에 있어서,

[0226] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0227] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0228] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 세포.

[0229] 85. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 형광 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터; 및

[0230] (b) (ii) 형광 단백질의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터

[0231] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 형광 단백질의 C-말단 단편으로의 형광 단백질의 N-말단 단편의 연결을 촉매하여 전장 형광 단백질을 생성하는 것인 진핵생물 세포.

[0232] 86. 단락 85에 있어서, 트랜스제닉 진핵생물 세포를 생성하기 위해 진핵생물 세포로의 제1 및 제2 벡터의 도입을 허용하는 조건 하에 진핵생물 세포를 유지하는 것을 추가로 포함하는 세포.

[0233] 87. 단락 86에 있어서, 전장 형광 단백질을 포함하는 트랜스제닉 진핵생물 세포를 선택하는 것을 추가로 포함하는 세포.

[0234] 88. 단락 85 내지 87 중 어느 한 단락에 있어서, 진핵생물 세포가 포유동물 세포인 세포.

[0235] 89. 단락 85 내지 88 중 어느 한 단락에 있어서, 형광 단백질이 TagCFP, mTagCFP2, Czurite, ECFP2, mKalama1, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3C, mTurquoise, mTurquoise2, 모노머 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, 모노머 Czami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, 모노머 Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mCpple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, TagRFP657, IFP1.4 및 iRFP로부터 선택된 것인 세포.

[0236] 90. 단락 85 내지 89 중 어느 한 단락에 있어서, 인테인이 분할 인테인인 세포.

[0237] 91. 단락 90에 있어서, 분할 인테인이 자연 분할 인테인인 세포.

[0238] 92. 단락 91에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 세포.

[0239] 93. 단락 92에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 세포.

[0240] 94. 단락 93에 있어서, 분할 인테인이 조작된 분할 인테인인 세포.

[0241] 95. 단락 94에 있어서, 조작된 분할 인테인이 DnaB 인테인으로부터 조작된 것인 세포.

[0242] 96. 단락 95에 있어서, 조작된 분할 인테인이 SspDnaB S1 인테인인 세포.

[0243] 97. 단락 95에 있어서, 조작된 분할 인테인이 GyrB 인테인으로부터 조작된 것인 세포.

[0244] 98. 단락 97에 있어서, 조작된 분할 인테인이 SspGyrB S11 인테인인 세포.

[0245] 99. 단락 85 내지 98 중 어느 한 단락에 있어서, 제1 및/또는 제2 분자가 단백질인 세포.

[0246] 100. 단락 85 내지 99 중 어느 한 단락에 있어서, 제1 및/또는 제2 분자가 비-코딩 리보핵산 (RNA)인 세포.

[0247] 101. 단락 100에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 세포.

[0248] 102. 단락 85 내지 101 중 어느 한 단락에 있어서, 제1 및/또는 제2 벡터가 플라스미드 벡터 또는 바이러스 벡터인 세포.

[0249] 103. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, egfp 유전자의 N-말단

단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0250] (b) (ii) egfp 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0251] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 egfp 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 egfp 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 EGFP 단백질을 생성하는 것인 진핵생물 세포.

[0252] 104. 단락 103에 있어서, egfp 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 5의 아미노산 1-175에 의해 확인된 아미노산 서열을 포함하고, egfp 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 5의 아미노산 175-239에 의해 확인된 아미노산 서열을 포함하는 것인 세포.

[0253] 105. 단락 103 또는 104에 있어서,

[0254] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0255] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0256] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 세포.

[0257] 106. (a) (i) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, mScarlet 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 포함하는 제1 벡터; 및

[0258] (b) (ii) mScarlet 유전자의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 포함하는 제2 벡터

[0259] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 mScarlet 단백질을 생성하는 것인 진핵생물 세포.

[0260] 107. 단락 106에 있어서,

[0261] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-46에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 47-232에 의해 확인된 아미노산 서열을 포함하거나;

[0262] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-48에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 49-232에 의해 확인된 아미노산 서열을 포함하거나;

[0263] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-51에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 52-232에 의해 확인된 아미노산 서열을 포함하거나;

[0264] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-75에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 76-232에 의해 확인된 아미노산 서열을 포함하거나;

[0265] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-122에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 123-232에 의해 확인된 아미노산 서열을 포함하거나;

[0266] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-140에 의해 확인된 아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 141-232에 의해 확인된 아미노산 서열을 포함하거나;

[0267] mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 1-163에 의해 확인된

아미노산 서열을 포함하고, mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편이 서열식별번호: 6의 아미노산 164-232에 의해 확인된 아미노산 서열을 포함하는 것인 세포.

[0268] 108. 단락 106 또는 107에 있어서,

[0269] 인테인의 N-말단 단편이 서열식별번호: 16에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 17에 의해 확인되거나;

[0270] 인테인의 N-말단 단편이 서열식별번호: 7에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 8에 의해 확인되거나; 또는

[0271] 인테인의 N-말단 단편이 서열식별번호: 18 또는 서열식별번호: 9에 의해 확인되고, 인테인의 C-말단 단편이 서열식별번호: 19 또는 서열식별번호: 10에 의해 확인된 것인 세포.

[0272] 109. 단락 85 내지 108 중 어느 한 단락의 세포를 포함하는 조성물.

[0273] 110. (a) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터; 및

[0274] (b) 항생제 내성 단백질의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터

[0275] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 N-말단 단편 및 C-말단 단편의 연결을 촉매하여 전장 항생제 내성 단백질을 생성하는 것인 키트.

[0276] 111. 단락 110에 있어서, 항생제 내성 단백질이 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1 또는 블라스티시딘에 대한 내성을 부여하는 것인 키트.

[0277] 112. (a) 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 형광 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터; 및

[0278] (b) 형광 단백질의 C-말단 단편으로부터 상류에 있는, 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터

[0279] 를 포함하며, 여기서 인테인의 N-말단 단편 및 C-말단 단편은 형광 단백질의 C-말단 단편으로의 형광 단백질의 N-말단 단편의 연결을 촉매하여 전장 형광 단백질을 생성하는 것인 키트.

[0280] 113. 단락 112에 있어서, 형광 단백질이 TagCFP, mTagCFP2, Czurite, ECFP2, mKalama1, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3C, mTurquoise, mTurquoise2, 모노머 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, 모노머 Czami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, 모노머 Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mCpple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, TagRFP657, IFP1.4 및 iRFP로부터 선택된 것인 키트.

[0281] 114. 단락 110 내지 113 중 어느 한 단락에 있어서, 인테인이 분할 인테인인 키트.

[0282] 115. 단락 114에 있어서, 분할 인테인이 자연 분할 인테인 또는 조작된 분할 인테인인 키트.

[0283] 116. 단락 115에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 키트.

[0284] 117. 단락 116에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 키트.

[0285] 118. 단락 115에 있어서, 조작된 분할 인테인이 DnaB 인테인 또는 GyrB 인테인으로부터 조작된 것인 키트.

[0286] 119. 단락 118에 있어서, 조작된 분할 인테인이 SspDnaB S1 인테인인 키트.

[0287] 120. 단락 118에 있어서, 조작된 분할 인테인이 SspGyrB S11 인테인인 키트.

[0288] 121. 단락 112 내지 120 중 어느 한 단락에 있어서, 다음 구성성분: 완충제, 염, 클로닝 효소, 감응성 세포, 형질감염 시약, 항생제, 및/또는 본원에 기재된 방법의 수행에 대한 지침서 중 임의의 하나 이상을 추가로 포함하는 키트.

[0289] 122. (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질

의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터,

[0290] (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및

[0291] (c) (i) 항생제 내성 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터

[0292] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 중심 단편으로의 항생제 내성 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 C-말단 단편으로의 항생제 내성 단백질의 중심 단편의 연결을 촉매하여 전장 항생제 내성 단백질을 생성하는 것인 방법.

[0293] 123. 단락 112에 있어서, 트랜스제닉 진핵생물 세포를 생성하기 위해 진핵생물 세포로의 제1, 제2 및 제3 벡터의 도입을 허용하는 조건 하에 진핵생물 세포를 유지하는 것을 추가로 포함하는 방법.

[0294] 124. 단락 123에 있어서, 전장 항생제 내성 단백질을 포함하는 트랜스제닉 진핵생물 세포를 선택하는 것을 추가로 포함하는 방법.

[0295] 125. 단락 112 내지 124 중 어느 한 단락에 있어서, 진핵생물 세포가 포유동물 세포인 방법.

[0296] 126. 단락 112 내지 125 중 어느 한 단락에 있어서, 항생제 내성 단백질이 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1 또는 블라스티시딘에 대한 내성을 부여하는 것인 방법.

[0297] 127. 단락 126에 있어서, 항생제 내성 단백질이 히그로마이신에 대한 내성을 부여하는 것인 방법.

[0298] 128. 단락 112 내지 127 중 어느 한 단락에 있어서, 제1 인테인이 분할 인테인인 방법.

[0299] 129. 단락 112 내지 128 중 어느 한 단락에 있어서, 제2 인테인이 분할 인테인인 방법.

[0300] 130. 단락 128 또는 129에 있어서, 분할 인테인이 자연 분할 인테인인 방법.

[0301] 131. 단락 130에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 방법.

[0302] 132. 단락 131에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 방법.

[0303] 133. 단락 132에 있어서, 제1 인테인이 NpuDnaE 인테인이고 제2 인테인이 NpuDnaE 인테인인 방법.

[0304] 134. 단락 112 내지 133 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 단백질인 방법.

[0305] 135. 단락 112 내지 133 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 비-코딩 리보핵산 (RNA)인 방법.

[0306] 136. 단락 135에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 방법.

[0307] 137. 단락 112 내지 136 중 어느 한 단락에 있어서, 제1 벡터, 제2 벡터, 제3 벡터 또는 이들의 임의의 조합이 플라스미드 벡터 또는 바이러스 벡터인 방법.

[0308] 138. (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, hygB 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터,

[0309] (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, hygB 유전자의 중심 단편으로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및

[0310] (c) (i) hygB 유전자의 C-말단 단편으로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터

[0311] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 hygB 유전자의 중심 단편에 의해 코딩된 단백질 단편으로의 hygB 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 hygB 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 hygB 유전자의 중심 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 히그로마이신 B 포스포트랜스퍼라제를 생성하는 것인 방법.

[0312] 139. 단락 138에 있어서, 제1 벡터가 서열식별번호: 29에 의해 확인된 서열을 코딩하고, 제2 벡터가 서열식별번호: 61에 의해 확인된 서열을 코딩하고, 제3 벡터가 서열식별번호: 23에 의해 확인된 서열을 코딩하는 것인 방법.

[0313] 140. 단락 138에 있어서, 제1 벡터가 서열식별번호: 21에 의해 확인된 서열을 코딩하고, 제2 벡터가 서열식별번호: 61에 의해 확인된 서열을 코딩하고, 제3 벡터가 서열식별번호: 35에 의해 확인된 서열을 코딩하는 것인 방법.

[0314] 141. (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터,

[0315] (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및

[0316] (c) (i) 항생제 내성 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터

[0317] 를 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 중심 단편으로의 항생제 내성 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 C-말단 단편으로의 항생제 내성 단백질의 중심 단편의 연결을 촉매하여 전장 항생제 내성 단백질을 생성하는 것인 진핵생물 세포.

[0318] 142. 단락 112에 있어서, 진핵생물 세포가 포유동물 세포인 진핵생물 세포.

[0319] 143. 단락 141 또는 142에 있어서, 항생제 내성 단백질이 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1 또는 블라스티시딘에 대한 내성을 부여하는 것인 진핵생물 세포.

[0320] 144. 단락 143에 있어서, 항생제 내성 단백질이 히그로마이신에 대한 내성을 부여하는 것인 진핵생물 세포.

[0321] 145. 단락 141 내지 144 중 어느 한 단락에 있어서, 제1 인테인이 분할 인테인인 진핵생물 세포.

[0322] 146. 단락 142 내지 145 중 어느 한 단락에 있어서, 제2 인테인이 분할 인테인인 진핵생물 세포.

[0323] 147. 단락 145 또는 146에 있어서, 분할 인테인이 자연 분할 인테인인 진핵생물 세포.

[0324] 148. 단락 147에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 진핵생물 세포.

[0325] 149. 단락 148에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 진핵생물 세포.

[0326] 150. 단락 149에 있어서, 제1 인테인이 NpuDnaE 인테인이고 제2 인테인이 NpuDnaE 인테인인 진핵생물 세포.

[0327] 151. 단락 142 내지 150 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 단백질인 진핵생물 세포.

[0328] 152. 단락 142 내지 150 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 비-코딩 리보핵산 (RNA)인 진핵생물 세포.

[0329] 153. 단락 152에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 진핵생물 세포.

[0330] 154. 단락 142 내지 153 중 어느 한 단락에 있어서, 제1 벡터, 제2 벡터, 제3 벡터 또는 이들의 임의의 조합이

플라스미드 벡터 또는 바이러스 벡터인 진핵생물 세포.

[0331] 155. 단락 142 내지 154 중 어느 한 단락의 진핵생물 세포를 포함하는 조성물.

[0332] 156. (a) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터,

[0333] (b) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 항생제 내성 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및

[0334] (c) 항생제 내성 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터

[0335] 를 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 중심 단편으로의 항생제 내성 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 항생제 내성 단백질의 C-말단 단편으로의 항생제 내성 단백질의 중심 단편의 연결을 촉매하여 전장 항생제 내성 단백질을 생성하는 것인 키트.

[0336] 157. 단락 156에 있어서, 항생제 내성 단백질이 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1 또는 블라스티시딘에 대한 내성을 부여하는 것인 키트.

[0337] 158. 단락 157에 있어서, 항생제 내성 단백질이 히그로마이신에 대한 내성을 부여하는 것인 키트.

[0338] 159. 단락 156 내지 158 중 어느 한 단락에 있어서, 제1 인테인이 분할 인테인인 키트.

[0339] 160. 단락 156 내지 159 중 어느 한 단락에 있어서, 제2 인테인이 분할 인테인인 키트.

[0340] 161. 단락 159 또는 160에 있어서, 분할 인테인이 자연 분할 인테인인 키트.

[0341] 162. 단락 161에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 키트.

[0342] 163. 단락 162에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 키트.

[0343] 164. 단락 163에 있어서, 제1 인테인이 NpuDnaE 인테인이고 제2 인테인이 NpuDnaE 인테인인 키트.

[0344] 165. 단락 156 내지 164 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 단백질인 키트.

[0345] 166. 단락 156 내지 164 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 비-코딩 리보핵산 (RNA)인 키트.

[0346] 167. 단락 166에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 키트.

[0347] 168. 단락 156 내지 167 중 어느 한 단락에 있어서, 제1 벡터, 제2 벡터, 제3 벡터 또는 이들의 임의의 조합이 플라스미드 벡터 또는 바이러스 벡터인 키트.

[0348] 169. (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 형광 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터,

[0349] (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 형광 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및

[0350] (c) (i) 형광 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터

[0351] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 형광 단백질의 중심 단편으로의 형광 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편

은 형광 단백질의 C-말단 단편으로의 형광 단백질의 중심 단편의 연결을 촉매하여 전장 형광 단백질을 생성하는 것인 방법.

[0352] 170. 단락 169에 있어서, 트랜스제닉 진핵생물 세포를 생성하기 위해 진핵생물 세포로의 제1, 제2 및 제3 벡터의 도입을 허용하는 조건 하에 진핵생물 세포를 유지하는 것을 추가로 포함하는 방법.

[0353] 171. 단락 170에 있어서, 전장 형광 단백질을 포함하는 트랜스제닉 진핵생물 세포를 선택하는 것을 추가로 포함하는 방법.

[0354] 172. 단락 169 내지 171 중 어느 한 단락에 있어서, 진핵생물 세포가 포유동물 세포인 방법.

[0355] 173. 단락 169 내지 172 중 어느 한 단락에 있어서, 형광 단백질이 TagCFP, mTagCFP2, Czurite, ECFP2, mKalama1, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3C, mTurquoise, mTurquoise2, 모노머 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, 모노머 Czami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, 모노머 Kusabira-Orange, mKOκ, mScarlet, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mCpple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, TagRFP657, IFP1.4 및 iRFP로부터 선택된 것인 방법.

[0356] 174. 단락 173에 있어서, 형광 단백질이 mScarlet인 방법.

[0357] 175. 단락 169 내지 174 중 어느 한 단락에 있어서, 제1 인테인이 분할 인테인인 방법.

[0358] 176. 단락 169 내지 175 중 어느 한 단락에 있어서, 제2 인테인이 분할 인테인인 방법.

[0359] 177. 단락 175 또는 176에 있어서, 분할 인테인이 자연 분할 인테인인 방법.

[0360] 178. 단락 177에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 방법.

[0361] 179. 단락 178에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 방법.

[0362] 180. 단락 179에 있어서, 제1 인테인이 NpuDnaE 인테인이고 제2 인테인이 NpuDnaE 인테인인 방법.

[0363] 181. 단락 169 내지 170 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 단백질인 방법.

[0364] 182. 단락 169 내지 180 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 비-코딩 리보핵산 (RNA)인 방법.

[0365] 183. 단락 182에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 방법.

[0366] 184. 단락 169 내지 183 중 어느 한 단락에 있어서, 제1 벡터, 제2 벡터, 제3 벡터 또는 이들의 임의의 조합이 플라스미드 벡터 또는 바이러스 벡터인 방법.

[0367] 185. (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, mScarlet 유전자의 N-말단 단편 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터,

[0368] (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, mScarlet 유전자의 중심 단편으로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및

[0369] (c) (i) mScarlet 유전자의 C-말단 단편으로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터

[0370] 를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 mScarlet 유전자의 중심 단편에 의해 코딩된 단백질 단편으로의 mScarlet 유전자의 N-말단 단편에 의해 코딩된 단백질 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 mScarlet 유전자의 C-말단 단편에 의해 코딩된 단백질 단편으로의 mScarlet 유전자의 중심 단편에 의해 코딩된 단백질 단편의 연결을 촉매하여 전장 mScarlet 단백질을 생성하는 것인 방법.

[0371] 186. 단락 185에 있어서, 제1 벡터가 서열식별번호: 121에 의해 확인된 서열을 코딩하고, 제2 벡터가 서열식별번호: 123에 의해 확인된 서열을 코딩하고, 제3 벡터가 서열식별번호: 125에 의해 확인된 서열을 코딩하는 것인 방법.

[0372] 187. (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 형광 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터,

[0373] (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 형광 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및

[0374] (c) (i) 형광 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터

[0375] 를 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 형광 단백질의 중심 단편으로의 형광 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 형광 단백질의 C-말단 단편으로의 형광 단백질의 중심 단편의 연결을 촉매하여 전장 형광 단백질을 생성하는 것인 진핵생물 세포.

[0376] 188. 단락 187에 있어서, 진핵생물 세포가 포유동물 세포인 진핵생물 세포.

[0377] 189. 단락 187 또는 188에 있어서, 형광 단백질이 TagCFP, mTagCFP2, Czurite, ECFP2, mKalama1, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3C, mTurquoise, mTurquoise2, 모노머 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, 모노머 Czami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, 모노머 Kusabira-Orange, mKOκ, mScarlet, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mCpple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, TagRFP657, IFP1.4 및 iRFP로부터 선택된 것인 진핵생물 세포.

[0378] 190. 단락 189에 있어서, 형광 단백질이 mScarlet인 진핵생물 세포.

[0379] 191. 단락 187 내지 190 중 어느 한 단락에 있어서, 제1 인테인이 분할 인테인인 진핵생물 세포.

[0380] 192. 단락 185 내지 191 중 어느 한 단락에 있어서, 제2 인테인이 분할 인테인인 진핵생물 세포.

[0381] 193. 단락 191 또는 192에 있어서, 분할 인테인이 자연 분할 인테인인 진핵생물 세포.

[0382] 194. 단락 193에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 진핵생물 세포.

[0383] 195. 단락 194에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 진핵생물 세포.

[0384] 196. 단락 195에 있어서, 제1 인테인이 NpuDnaE 인테인이고 제2 인테인이 NpuDnaE 인테인인 진핵생물 세포.

[0385] 197. 단락 185 내지 196 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 단백질인 진핵생물 세포.

[0386] 198. 단락 185 내지 196 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 비-코딩 리보핵산 (RNA)인 진핵생물 세포.

[0387] 199. 단락 198에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 진핵생물 세포.

[0388] 200. 단락 185 내지 199 중 어느 한 단락에 있어서, 제1 벡터, 제2 벡터, 제3 벡터 또는 이들의 임의의 조합이 플라스미드 벡터 또는 바이러스 벡터인 진핵생물 세포.

[0389] 201. 단락 185 내지 200 중 어느 한 단락의 진핵생물 세포를 포함하는 조성물.

[0390] 202. (a) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 형광 단백질의 N-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터,

[0391] (b) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 형광 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및

[0392] (c) 형광 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터

[0393] 를 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 형광 단백질의 중심 단편으로의 형광 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 형광 단백질의 C-말단 단편으로의 형광 단백질의 중심 단편의 연결을 촉매하여 전장 형광 단백질을 생성하는 것인 키트.

[0394] 203. 단락 202에 있어서, 형광 단백질이 TagCFP, mTagCFP2, Czurite, ECFP2, mKalama1, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3C, mTurquoise, mTurquoise2, 모노머 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, 모노머 Czami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, 모노머 Kusabira-Orange, mKOκ, mScarlet, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mCpple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, TagRFP657, IFP1.4 및 iRFP로부터 선택된 것인 키트.

[0395] 204. 단락 203에 있어서, 형광 단백질이 mScarlet인 키트.

[0396] 205. 단락 202 내지 204 중 어느 한 단락에 있어서, 제1 인테인이 분할 인테인인 키트.

[0397] 206. 단락 202 내지 205 중 어느 한 단락에 있어서, 제2 인테인이 분할 인테인인 키트.

[0398] 207. 단락 206에 있어서, 분할 인테인이 자연 분할 인테인인 키트.

[0399] 208. 단락 207에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 키트.

[0400] 209. 단락 208에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 키트.

[0401] 210. 단락 209에 있어서, 제1 인테인이 NpuDnaE 인테인이고 제2 인테인이 NpuDnaE 인테인인 키트.

[0402] 211. 단락 202 내지 210 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 단백질인 키트.

[0403] 212. 단락 202 내지 210 중 어느 한 단락에 있어서, 관심있는 제1 분자, 관심있는 제2 분자, 관심있는 제3 분자 또는 이들의 임의의 조합이 비-코딩 리보핵산 (RNA)인 키트.

[0404] 213. 단락 212에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 또는 짧은-헤어핀 RNA (shRNA)인 키트.

[0405] 214. 단락 202 내지 213 중 어느 한 단락에 있어서, 제1 벡터, 제2 벡터, 제3 벡터 또는 이들의 임의의 조합이 플라스미드 벡터 또는 바이러스 벡터인 키트.

[0406] 215. 단락 202 내지 214 중 어느 한 단락에 있어서, 다음 구성성분: 완충제, 염, 클로닝 효소, 감응성 세포, 형질감염 시약, 항생제, 및/또는 본원에 기재된 방법의 수행에 대한 지침서 중 임의의 하나 이상을 추가로 포함하는 키트.

[0407] 216. (a) (i) N-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 선택가능한 마커 단백질 단편 (예를 들어, 항생제 내성 단백질 단편 또는 형광 단백질 단편)을 코딩하는 뉴클레오티드 서열 및 (ii) 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터, 및 (b) (i) 제2 선택가능한 마커 단백질 단편 (예를 들어, 항생제 내성 단백질 단편 또는 형광 단백질 단편)으로부터 상류에 있는, C-말단 인테인 단백질 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터를 진핵생물 세포를 포함하는 조성물로 전달하는 것을 포함하며, 여기서 N-말단 인테인 단백질 단편 및 C-말단 인테인 단백질 단편은 제2 선택가능한 마커 단백질 단편으로의 제1 선택가능한 마커 단백질 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성하는 것인 트랜스제닉 선택 방법.

[0408] 217. (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질 (예를 들어, 항생제 내성 단백질 또는 형광 단백질)의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii)

관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터, (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, 및 (c) (i) 선택가능한 마커 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 중심 단편으로의 선택가능한 마커 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 C-말단 단편으로의 선택가능한 마커 단백질의 중심 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성하는 것인 트랜스제닉 선택 방법.

[0409] 218. (a) (i) 제1 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질 (예를 들어, 항생제 내성 단백질 또는 형광 단백질)의 N-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제1 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제1 벡터, (b) (i) 제2 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 제1 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제1 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제2 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제2 벡터, (c) (i) 제3 인테인의 N-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 선택가능한 마커 단백질의 제2 중심 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제2 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제3 벡터, 및 (d) (i) 선택가능한 마커 단백질의 C-말단 단편을 코딩하는 뉴클레오티드 서열로부터 상류에 있는, 제3 인테인의 C-말단 단편을 코딩하는 뉴클레오티드 서열 및 (ii) 관심있는 제3 분자를 코딩하는 뉴클레오티드 서열을 포함하는 제4 벡터를 진핵생물 세포로 전달하는 것을 포함하며, 여기서 제1 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 제1 중심 단편으로의 선택가능한 마커 단백질의 N-말단 단편의 연결을 촉매하고, 제2 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 제2 중심 단편으로의 선택가능한 마커 단백질의 제1 중심 단편의 연결을 촉매하고, 제3 인테인의 N-말단 단편 및 C-말단 단편은 선택가능한 마커 단백질의 C-말단 단편으로의 선택가능한 마커 단백질의 제2 중심 단편의 연결을 촉매하여 전장 선택가능한 마커 단백질을 생성하는 것인 트랜스제닉 선택 방법.

[0410] 219. 단락 216 내지 218 중 어느 한 단락에 있어서, 트랜스제닉 진핵생물 세포를 생성하기 위해 진핵생물 세포로의 벡터의 도입을 허용하는 조건 하에 진핵생물 세포를 유지하는 것을 추가로 포함하는 방법.

[0411] 220. 단락 219에 있어서, 전장 선택가능한 마커 단백질을 포함하는 트랜스제닉 진핵생물 세포를 선택하는 것을 추가로 포함하는 방법.

[0412] 221. 단락 216 내지 220 중 어느 한 단락에 있어서, 진핵생물 세포가 포유동물 세포인 방법.

[0413] 222. 단락 216 내지 221 중 어느 한 단락에 있어서, 항생제 내성 단백질이 히그로마이신, G418, 퓨로마이신, 플레오마이신 D1 또는 블라스티시딘에 대한 내성을 부여하는 것인 방법.

[0414] 223. 단락 216 내지 222 중 어느 한 단락에 있어서, 인테인이 분할 인테인인 방법.

[0415] 224. 단락 223에 있어서, 분할 인테인이 자연 분할 인테인인 방법.

[0416] 225. 단락 224에 있어서, 자연 분할 인테인이 DnaE 인테인으로부터 선택된 것인 방법.

[0417] 226. 단락 225에 있어서, DnaE 인테인이 시네코시스티스 sp. DnaE (SspDnaE) 인테인 및 노스톡 푼크티포르메 (NpuDnaE) 인테인으로부터 선택된 것인 방법.

[0418] 227. 단락 223에 있어서, 분할 인테인이 조작된 분할 인테인인 방법.

[0419] 228. 단락 2278에 있어서, 조작된 분할 인테인이 DnaB 인테인으로부터 조작된 것인 방법.

[0420] 229. 단락 228에 있어서, 조작된 분할 인테인이 SspDnaB S1 인테인인 방법.

[0421] 230. 단락 229에 있어서, 조작된 분할 인테인이 GyrB 인테인으로부터 조작된 것인 방법.

[0422] 231. 단락 230에 있어서, 조작된 분할 인테인이 SspGyrB S11 인테인인 방법.

[0423] 232. 단락 216 내지 231 중 어느 한 단락에 있어서, 분자가 단백질로부터 선택된 것인 방법.

[0424] 233. 단락 216 내지 231 중 어느 한 단락에 있어서, 분자가 비-코딩 리보핵산 (RNA)으로부터 선택된 것인 방법.

[0425] 234. 단락 233에 있어서, 비-코딩 RNA가 마이크로RNA (miRNA), 안티센스 RNA, 짧은-간섭 RNA (siRNA) 및 짧은-헤어핀 RNA (shRNA)로부터 선택된 것인 방법.

[0426] 235. 단락 216 내지 234 중 어느 한 단락에 있어서, 벡터가 플라스미드 벡터 및 바이러스 벡터로부터 선택된 것인 방법.

[0427] <실시예>

[0428] 본 개시내용은 하기 실시예에 의해 추가로 예시된다. 이들 실시예는 본 개시내용의 이해를 돕기 위해 제공되며, 그의 제한으로 해석되어서는 안된다.

[0429] 실시예 1. 항생제 내성 마커

[0430] 선택가능한 마커는 종종 원하는 유전자형을 갖는 세포를 단리하기 위해 유전자 조작에 사용된다 [1]. 그러나, 진핵생물 세포에서 사용하기 위한 잘-특징화된 항생제 내성 유전자의 수는 제한되어 있고, 스펙트럼이 일반 실험실에서 장비에 의해 명백하게 식별될 수 있는 형광 단백질의 수는 제한되어 있다. 연구원은 종종 다중 트랜스진을 세포로 혼입시키는 경우 선택가능한 마커를 충분히 선택하지 못하는 문제를 겪는다. 다른 한편, 다중 항생제를 동시에 선택하는 것은 종종 세포에 가혹하다. "선택가능한 마커 재순환"은 제2의 해결책(work-around)을 제공할 수 있으나, 선택 마커의 트랜스제네시스, 선택 및 제거의 다수의 라운드를 필요로 한다 [2]. 다중 트랜스진이 하나의 선택 방식에 의해 동시에 선택되도록 하기 위해, 본 발명자들은 분할 항생제 내성 및 형광 단백질 유전자를 생성하였으며, 여기서 항생제 내성 또는 형광 단백질을 코딩하는 유전자는 단백질 트랜스-스플라이싱에 의해 재연결될 수 있는 인테인 ("마커트론")으로 융합된 2개 이상의 세그먼트로 분할된다 [3] (도 1a). 각각의 마커트론은 특정 트랜스진을 보유하는 트랜스제닉 벡터로 삽입된다. 마커트론의 세트를 함유하는 트랜스제닉 벡터의 전달은 마커트론의 서브세트 또는 완전한 세트를 보유하는 세포를 생성한다. 마커트론의 완전한 세트를 함유하는 세포만이 단백질 스플라이싱을 통해 완전히 재구성된 마커 단백질을 생성하므로 선택을 통해 통과하는 반면, 마커트론의 부분 세트를 갖는 세포는 제거되어, 모든 의도된 트랜스진을 함유하는 세포의 공동선택을 달성한다.

[0431] 본 발명자들은 이중 트랜스제네시스를 위한 2-마커트론 인테인-분할 내성 (Intres) 유전자의 조작으로 시작하였다. 플랭킹 잔기 및 국소 단백질 폴딩은 인테인-매개 트랜스-스플라이싱의 효율에 영향을 미칠 수 있기 때문에, 본 발명자들은 NpuDnaE [4, 5] 및 SspDnaB [6]로부터 유래된 2개의 잘-특징화된 분할 인테인과 호환가능한 4개의 통상적으로 사용되는 항생제 내성 유전자 각각에서 분할점을 확인하는 것을 시작하였다. 이중 트랜스제닉 선택의 유효성의 평가를 용이하게 하기 위해, 본 발명자들은 시험 트랜스진으로서 TagBFP2 또는 mCherry 형광 단백질을 발현하는 렌티바이러스 벡터 상에 마커트론을 클로닝하였다 (도 1b). 바이러스 제제를 U2OS 세포로 형질도입한 후, 비-선택 또는 선택 배지를 갖는 복제 플레이트로 분할하였다. 항생제 선택을 위한 적절한 계대배양 후, 2개의 세포 배양물을 유동 세포계측법에 의해 분석하였다. 히그로마이신 (Hygro) 내성 유전자의 경우, 플랭킹 잔기 "GS"를 갖는 하나의 "천연" SspDnaB 분할점 (G200:S201) 및 "YC" 잔기를 갖는 하나의 "천연" NpuDnaE 분할점 (Y89:C90)을 시험하였다. 둘 모두는 N- 및 C-마커트론 둘 모두가 형질도입될 때 성공적인 선택을 가능하게 하였으며, 비-선택된 배양물에서 <10% 이중-양성 세포와 비교하여 선택된 배양물에서 >99% BFP+ mCherry+ 이중 트랜스제닉 세포를 얻었다 (도 3; 플라스미드 쌍 3,4 및 5,6). 2개의 마커트론 중 하나로 형질도입된 세포는 히그로마이신 선택에서 생존하지 않았다. 대조적으로, 통상적인 전장 비-분할 히그로마이신 벡터를 사용한 이중 트랜스제네시스는 BFP+ mCherry+ 세포의 약 20% 풍부만을 허용하였다 (플라스미드 쌍 97,98). 본 발명자들은 C-익스테인 이음부 상의 필수 시스테인 잔기 및 이전 보고서 7에서 실질적인 트랜스-스플라이싱 활성을 지지하는 N-익스테인 이음부 상의 잔기를 갖는 NpuDnaE에 대한 3개의 추가 잠재적인 분할점 (52S:53C), (240A:241C) 및 (292R:293C)를 스크리닝하였다. 또한, 본 발명자들은 추가 분할점을 생성하는 전위성 부위에서 스플라이싱을 지지하기 위해 C-익스테인 이음부 상에 "인공" 시스테인을 삽입함으로써 6개의 추가 NpuDnaE 분할점을 혼입하였다. 전체로서, 시험된 11개의 분할점 중 8개는 히그로마이신 선택을 지지하였다 (도 3). 유사하게, 퓨로마이신 (Puro) (도 4), 네오마이신 (Neo) (도 5) 및 블라스티시딘 (Blast) (도 6) 내성 유전자의 경우, 본 발명자들은 4, 2 및 1개의 기능성 Intres 쌍(들)을 각각 확인하였다. 이들 모든 경우에, 마커트론으로 형질도입된 세포는 선택에서 생존하지 않았으나, 둘 모두로 형질도입된 세포는 블라스티시딘(102) Intres를 제외하고 비-선택 배양물에서 <50%와 비교하여 선택 배양물에서 >95% 이중 트랜스제닉 세포를 수득하였으며, 91% 이중 트랜스제닉 세포의 더 낮지만 여전히 유의하게 풍부함을 달성하였다 (도 3-6). Intres 유전

자 및 플라스미드의 분할점의 세부사항은 도 2a-2d 및 표 1에 제공된다.

표 1. 플라스미드

플라스미드 #*	플라스미드 명칭	마커트론
3	pLX-Hygro(1-89)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-89)-NpuDnaE(N)
4	pLX-NpuDnaE(C)-Hygro(90-341)-IRES-mCherry	NpuDnaE(C)-Hygro(90-341)
5	pLX-Hygro(1-200)-SspDnaB(N)-IRES-TagBFP2	Hygro(1-200)-SspDnaB(N)
6	pLX-SspDnaB(C)-Hygro(201-341)-IRES-mCherry	SspDnaB(C)-Hygro(201-341)
7	pLX-Hygro(1-52)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-52)-NpuDnaE(N)
8	pLX-NpuDnaE(C)-Hygro(53-341)-IRES-mCherry	NpuDnaE(C)-Hygro(53-341)
9	pLX-Hygro(1-240)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-240)-NpuDnaE(N)
10	pLX-NpuDnaE(C)-Hygro(241-341)-IRES-mCherry	NpuDnaE(C)-Hygro(241-341)
11	pLX-Hygro(1-292)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-292)-NpuDnaE(N)
12	pLX-NpuDnaE(C)-Hygro(293-341)-IRES-mCherry	NpuDnaE(C)-Hygro(293-341)
13	pLX-Blast(1-102)-NpuDnaE(N)-IRES-TagBFP2	Blast(1-102)-NpuDnaE(N)
14	pLX-NpuDnaE(C)-Blast(103-140)-IRES-mCherry	NpuDnaE(C)-Blast(103-140)
17	pLX-Puro(1-119)-NpuDnaE(N)-IRES-TagBFP2	Puro(1-119)-NpuDnaE(N)
18	pLX-NpuDnaE(C)-Puro(insCys;120-199)-IRES-mCherry	NpuDnaE(C)-Puro(insCys;120-199)
19	pLX-Puro(1-100)-SspDnaB(N-S0)-IRES-TagBFP2	Puro(1-100)-SspDnaB(N-S0)
20	pLX-SspDnaB(C-S0)-Puro(101-199)-IRES-mCherry	SspDnaB(C-S0)-Puro(101-199)
21	pLX-Neo(1-133)-NpuDnaE(N)-IRES-TagBFP2	Neo(1-133)-NpuDnaE(N)
22	pLX-NpuDnaE(C)-Neo(134-267)-IRES-mCherry	NpuDnaE(C)-Neo(134-267)
23	pLX-Neo(1-194)-NpuDnaE(N)-IRES-TagBFP2	Neo(1-194)-NpuDnaE(N)
24	pLX-NpuDnaE(C)-Neo(195-267)-IRES-mCherry	NpuDnaE(C)-Neo(195-267)
25	pLX-NpuDnaE(C)_Hygro(53-89)-NpuDnaE(N)-IRES-GFP	NpuDnaE(C)_Hygro(53-89)-NpuDnaE(N)
26	pLX-NpuDnaE(C)_Hygro(53-239)-NpuDnaE(N)-IRES-GFP	NpuDnaE(C)_Hygro(53-239)-NpuDnaE(N)
27	pCR8-BsaI->ccdbCam<-BsaI-NpuDnaE(N)-MD1-68-15	
28	pCR8-NpuDnaE(C)_BsaI->ccdbCam<-BsaI-MD1-68-18	
29	pCR8-BsaI->ccdbCam<-BsaI-SspDnaE(N)-MD1-68-12	
30	pCR8-SspDnaE(C)_BsaI->ccdbCam<-BsaI-MD1-68-13	
31	pCR8-BsaI->ccdbCam<-BsaI-SspDnaB(N-S0)-25-135-18	
32	pCR8-SspDnaB(C-S0)_BsaI->ccdbCam<-BsaI-25-155-41	
33	pLX-mScarlet(1-46)-NpuDnaE(N)_LZA-IRES-TagBFP2	mScarlet(1-46)-NpuDnaE(N)_LZA
34	pLX-LZB_NpuDnaE(C)-mScarlet(insCys;47-232)-IRES-TagBFP2	LZB_NpuDnaE(C)-mScarlet(insCys;47-232)
35	pLX-mScarlet(1-48)-NpuDnaE(N)_LZA-IRES-TagBFP2	mScarlet(1-48)-NpuDnaE(N)_LZA
36	pLX-LZB_NpuDnaE(C)-mScarlet(insCys;49-232)-IRES-GFP	LZB_NpuDnaE(C)-mScarlet(insCys;49-232)
37	pLX-mScarlet(1-51)-NpuDnaE(N)_LZA -IRES-TagBFP2	mScarlet(1-51)-NpuDnaE(N)_LZA
38	pLX-LZB_NpuDnaE(C)-mScarlet(insCys;52-232)-IRES-GFP	LZB_NpuDnaE(C)-mScarlet(insCys;52-232)
39	pLX-mScarlet(1-75)-NpuDnaE(N)_LZA-IRES-TagBFP2	mScarlet(1-75)-NpuDnaE(N)_LZA
40	pLX-LZB_NpuDnaE(C)-mScarlet(insCys;76-232)-IRES-GFP	LZB_NpuDnaE(C)-mScarlet(insCys;76-232)
41	pLX-mScarlet(1-122)-NpuDnaE(N)_LZA-IRES-TagBFP2	mScarlet(1-122)-NpuDnaE(N)_LZA

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플라스미드 #*	플라스미드 명칭	마커트론
42	pLX-LZB_NpuDnaE(C)-mScarlet(insCys;123-232)-IRES-GFP	LZB_NpuDnaE(C)-mScarlet(insCys;123-232)
43	pLX-mScarlet(1-140)-NpuDnaE(N)_LZA-IRES-TagBFP2	mScarlet(1-140)-NpuDnaE(N)_LZA
44	pLX-LZB_NpuDnaE(C)-mScarlet(insCys;141-232)-IRES-GFP	LZB_NpuDnaE(C)-mScarlet(insCys;141-232)
45	pLX-mScarlet(1-163)-NpuDnaE(N)_LZA-IRES-TagBFP2	mScarlet(1-163)-NpuDnaE(N)_LZA
46	pLX-LZB_NpuDnaE(C)-mScarlet(insCys;164-232)-IRES-GFP	LZB_NpuDnaE(C)-mScarlet(insCys;164-232)
47	pCR8-TagBFP2	TagBFP2
48	pCR8-mCherry	mCherry
49	pLX-DEST-IRES-Hygro(1-89)-NpuDnaE(N)	Hygro(1-89)-NpuDnaE(N)
50	pLX-DEST-IRES-NpuDnaE(C)-Hygro(90-341)	NpuDnaE(C)-Hygro(90-341)
51	pLX-[TagBFP2]-IRES-Hygro(1-89)-NpuDnaE(N)	Hygro(1-89)-NpuDnaE(N)
52	pLX-[mCherry]-IRES-NpuDnaE(C)-Hygro(90-341)	NpuDnaE(C)-Hygro(90-341)
53	pLX-DEST-IRES-Puro(1-119)-NpuDnaE(N)	Puro(1-119)-NpuDnaE(N)
54	pLX-DEST-IRES-NpuDnaE(C)-Puro(120-199)	NpuDnaE(C)-Puro(insCys;120-199)
55	pLX-[TagBFP2]-IRES-Puro(1-119)-NpuDnaE(N)	Puro(1-119)-NpuDnaE(N)
56	pLX-[mCherry]-IRES-NpuDnaE(C)-Puro(120-199)	NpuDnaE(C)-Puro(insCys;120-199)
57	pLX-DEST-IRES-Neo(1-194)-NpuDnaE(N)	Neo(1-194)-NpuDnaE(N)
58	pLX-DEST-IRES-NpuDnaE(C)-Neo(195-267)	NpuDnaE(C)-Neo(195-267)
59	pLX-[TagBFP2]-IRES-Neo(1-194)-NpuDnaE(N)	Neo(1-194)-NpuDnaE(N)
60	pLX-[mCherry]-IRES-NpuDnaE(C)-Neo(195-267)	NpuDnaE(C)-Neo(195-267)
64	pLX-Hygro(1-69)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-69)-NpuDnaE(N)
65	pLX-NpuDnaE(C)-Hygro(^C;70-341)-IRES-mCherry	NpuDnaE(C)-Hygro(^C;70-341)
66	pLX-Hygro(1-131)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-131)-NpuDnaE(N)
67	pLX-NpuDnaE(C)-Hygro(^C;132-341)-IRES-mCherry	NpuDnaE(C)-Hygro(^C;132-341)
68	pLX-Hygro(1-171)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-171)-NpuDnaE(N)
69	pLX-NpuDnaE(C)-Hygro(^C;172-341)-IRES-mCherry	NpuDnaE(C)-Hygro(^C;172-341)
70	pLX-Hygro(1-218)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-218)-NpuDnaE(N)
71	pLX-NpuDnaE(C)-Hygro(^C;219-341)-IRES-mCherry	NpuDnaE(C)-Hygro(^C;219-341)
72	pLX-Hygro(1-259)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-259)-NpuDnaE(N)
73	pLX-NpuDnaE(C)-Hygro(^C;260-341)-IRES-mCherry	NpuDnaE(C)-Hygro(^C;260-341)
74	pLX-Hygro(1-277)-NpuDnaE(N)-IRES-TagBFP2	Hygro(1-277)-NpuDnaE(N)
75	pLX-NpuDnaE(C)-Hygro(^C; 278-341)-IRES-mCherry	NpuDnaE(C)-Hygro(^C;278-341)
76	pLX-Puro(1-32)-NpuDnaE(N)-IRES-TagBFP2	Puro(1-32)-NpuDnaE(N)
77	pLX-NpuDnaE(C)-Puro(^C;33-199)-IRES-mCherry	NpuDnaE(C)-Puro(^C;33-199)
78	pLX-Puro(1-84)-NpuDnaE(N)-IRES-TagBFP2	Puro(1-84)-NpuDnaE(N)
79	pLX-NpuDnaE(C)-Puro(^C;85-199)-IRES-mCherry	NpuDnaE(C)-Puro(^C;85-199)
80	pLX-Puro(1-137)-NpuDnaE(N)-IRES-TagBFP2	Puro(1-137)-NpuDnaE(N)
81	pLX-NpuDnaE(C)-Puro(^C;138-199)-IRES-mCherry	NpuDnaE(C)-Puro(^C;138-199)
82	pLX-Puro(1-158)-NpuDnaE(N)-IRES-TagBFP2	Puro(1-158)-NpuDnaE(N)
83	pLX-NpuDnaE(C)-Puro(^C;159-199)-IRES-mCherry	NpuDnaE(C)-Puro(^C;159-199)
84	pLX-Puro(1-180)-NpuDnaE(N)-IRES-TagBFP2	Puro(1-180)-NpuDnaE(N)
85	pLX-NpuDnaE(C)-Puro(^C;181-199)-IRES-mCherry	NpuDnaE(C)-Puro(^C;181-199)
86	pLX-Blast(1-58)-NpuDnaE(N)-IRES-TagBFP2	Blast(1-58)-NpuDnaE(N)
87	pLX-NpuDnaE(C)-Blast(59-140)-IRES-mCherry	NpuDnaE(C)-Blast(59-140)
88	pLX-NpuDnaE(C)-HygroBA-SspDnaB(N-S0)-IRES-EGFP	NpuDnaE(C)-Hygro(53-200)-SspDnaB(N-S0)
89	pLX-SspDnaB(C-S0)-Hygro(201-341)-IRES-mCherry	SspDnaB(C-S0)-Hygro(201-341)
90	pLX-NpuDnaE(C)-Hygro(90-200)-SspDnaB(N-S0)-IRES-EGFP	NpuDnaE(C)-Hygro(90-200)-SspDnaB(N-S0)
91	pLX-Hygro(1-200)-SspDnaB(N-S0)-IRES-TagBFP2	Hygro(1-200)-SspDnaB(N-S0)

[0433]

플라스미드 #*	플라스미드 명칭	마커트론
92	pLX-SspDnaB(C-S0)-Hygro(201-240)-NpuDnaE(N)-IRES-EGFP	SspDnaB(C-S0)-Hygro(201-240)-NpuDnaE(N)
93	pLX-SspDnaB(C-S0)-Hygro(201-292)-NpuDnaE(N)-IRES-EGFP	SspDnaB(C-S0)-Hygro(201-292)-NpuDnaE(N)
94	pLX-DEST-IRES-TagBFP2	
95	pLX-DEST-IRES-EGFP	
96	pLX-DEST-IRES-mCherry	
97	pLX-Hygro-IRES-TagBFP2	비-분할 Hygro
98	pLX-Hygro-IRES-mCherry	비-분할 Hygro
99	pLX-Puro-IRES-TagBFP2	비-분할 Puro
100	pLX-Puro-IRES-mCherry	비-분할 Puro
101	pLX-Hygro-IRES-EGFP	비-분할 Hygro
102	pLX-NLS_GFP-IRES-Hygro	비-분할 Hygro
103	pLX-LifeAct_mCherry-IRES-Hygro	비-분할 Hygro
104	pLX-NLS_GFP-IRES-Hygro(1-89)-NpuDnaE(N)	Hygro(1-89)-NpuDnaE(N)
105	pLX-LifeAct_mScarlet-IRES- NpuDnaE(C)-Hygro(90-341)	NpuDnaE(C)-Hygro(90-341)
106	pX330-AAVS1	
107	pAAVS1-Nst-EF1aHygro2ArtTA3(-)_TetO-Blast-P2A-EGFP	비-분할 Blast
108	pAAVS1-Nst-EF1aHygro2ArtTA3(-)_TetO-Blast-P2A-mScarlet	비-분할 Blast
109	pAAVS1-Nst-EF1aHygro2ArtTA3(-)_TetO-Blast(1-102)_NpuDnaE(N)-P2A-EGFP	Blast(1-102)-NpuDnaE(N)
110	pAAVS1-Nst-EF1aHygro2ArtTA3(-)_TetO-NpuDnaE(C)_Blast(103-140)-P2A-mScarlet	NpuDnaE(C)-Blast(103-140)
111	pAAVS1-Nst-EF1aBlast2ArtTA3(-)_TetO-Hygro-P2A-NTR-E2A-EGFP	비-분할 Hygro
112	pAAVS1-Nst-EF1aBlast2ArtTA3(-)_TetO-Hygro-P2A-NTR-E2A-mCherry	비-분할 Hygro
113	pAAVS1-Nst-EF1aBlast2ArtTA3(-)_TetO- Hygro(1-89)-NpuDnaE(N)-P2A-NTR-E2A-EGFP	Hygro(1-89)-NpuDnaE(N)
114	pAAVS1-Nst-EF1aBlast2ArtTA3(-)_TetO-NpuDnaE(C)-Hygro(90-341)-P2A-NTR-E2A-mCherry	NpuDnaE(C)-Hygro(90-341)
115	pLX-Hygro(1-89)_NpuDnaE(N)_LZA-IRES-TagBFP2	Hygro(1-89)-NpuDnaE(N)-LZA
116	pLX-LZB_NpuDnaGEP(C)_Hygro(90-200)_SspDnaB(N-S0)-IRES-GFP	LZB-NpuDnaGEP(C)-Hygro(90-200)-SspDnaB(N-S0)
117	pLX-SspDnaB(C-S0)_Hygro(201-240)_NpuDnaE(N)_LZA-IRES-GFP	SspDnaB(C-S0)-Hygro(201-240)-NpuDnaE(N)-LZA
118	pLX-LZB_NpuDnaGEP(C)_Hygro(241-341)-IRES-mCherry	LZB-NpuDnaGEP(C)-Hygro(241-341)

[0434]

[0435] 실시예 2. 게이트웨이-호환가능한 렌티바이러스 벡터

[0436] Intres 마커의 채택을 용이하게 하기 위해, 본 발명자들은 트랜스진 8의 편리한 제한-라이게이션-독립적 LR 클로나제 재조합을 위한 게이트웨이-호환가능한 렌티바이러스 벡터를 생성하였다 (도 7a). 본 발명자들은 TagBPF2 및 mCherry를 각각 N- 및 C-Intres 벡터에 재조합함으로써 이들 벡터의 기능성을 시험하였으며, 이중 트랜스제닉 세포의 강건한 선택을 발견하였다 (도 7b). Intres 벡터의 한 잠재적인 유용성은 세포에 상이한 형광 마커를 설치하여 상이한 세포 구획을 표지하는 것이다. 이러한 유용성을 탐색하기 위해, 본 발명자들은 통상적인 전장 (FL) 비-분할 히그로마이신 선택가능한 벡터 또는 2-마커트론 히그로마이신 Intres 벡터 및 플라스미드의 세트로 형질도입된 세포로의 게이트웨이 재조합, 이어서 항생제 선택에 의해, 핵 및 F-액틴을 각각 표지하는 NLS-GFP 및 LifeAct-mScarlet 9에서 클로닝하였다 (도 7c). 비-분할 선택가능한 플라스미드로 형질도입된 샘플은 단일 및 이중 표지된 세포 둘 모두를 함유한 반면, Intres 플라스미드로 형질도입된 세포는 모두 이중 표지되었다 (도 7c).

[0437] 실시예 3. 형광 마커

[0438] 분할 형광 마커가 트랜스진 선택을 위해 사용될 수 있는지를 시험하기 위해, 본 발명자들은 mScarlet 형광 단백질에 대한 NpuDnaE 분할점을 스크리닝하고 (도 8a), 4개의 분할점은 이중 트랜스제닉 세포의 >96% 풍부를 허용하고, 3개의 다른 분할점은 비-게이팅된 집단에서 <20% 이중 트랜스제닉 세포와 비교하여 mScarlet-게이팅된 집단에서 이중 트랜스제닉 세포의 >60% 풍부를 가능하게 한다는 것을 확인하였다 (도 8b).

[0439] 실시예 4. 고도의 분할 마커

[0440] 본 발명자들은 2-마커트론 Intres 유전자에 대해 확인된 분할점으로 고도의 분할 마커의 조작을 시작하였다. 본 발명자들은 하나의 항생제로 2개 초과의 "비연결된" 트랜스진의 공동선택을 허용하기 위해 마커 유전자를 3개 이상의 마커트론으로 분배하기 위해 분할점의 조합을 시험하였다 (도 9a-9b). 이러한 "Intres 쇄"를 허용하는 분할점의 쌍을 확인하기 위해, 본 발명자들은 3개의 형광 트랜스진 TagBFP2, EGFP 또는 mCherry 중 하나를

각각 보유하는 3개의 렌티바이러스 벡터로 3-분할 마커트론을 클로닝하였으며, 이는 본 발명자들이 유동 세포계측법에 의해 선택의 유효성을 평가하는 것을 가능하게 하였다 (도 9c). 히그로마이신 내성 유전자는 가장 길고, 시험을 위한 가장 큰 분할점을 제공하기 때문에, 본 발명자들은 3-마커트론 히그로마이신 Intres의 조작에 중점을 두었다. 본 발명자들은 2개의 개입 NpuDnaE 인테인을 사용하여 2개의 3-마커트론 히그로마이신 Intres를 시험하였으며, 제1 인테인을 위한 NpuDnaE 및 제2 인테인을 위한 SspDnaB를 사용할 뿐만 아니라 제1 인테인을 위한 SspDnaB 및 제2 인테인을 위한 NpuDnaE를 사용하였다 (도 9d). 비-선택된 배양물에서 <15% 삼중 트랜스제닉 세포와 비교하여, 이들 6개의 3-마커트론 히그로마이신 Intres 중 5개는 >97%를 가능하게 하였고, 나머지 1개는 히그로마이신-선택된 배양물에서 80% 삼중 트랜스제닉 선택을 가능하게 하였다. 리브-원-아웃 (leave-one-out) 형질도입을 갖는 샘플은 히그로마이신 선택 후 어떠한 생존가능 세포도 생성하지 않은 반면, 비-분할 히그로마이신 벡터로 형질도입된 세포는 선택 후 오직 7% 삼중 트랜스제닉 세포를 생성하였다.

[0441] 3-마커트론 Intres의 사용을 용이하게 하기 위해, 본 발명자들은 이들 마커를 갖는 게이트웨이 호환가능한 렌티바이러스 벡터를 생성하였다 (도 10a). TagBFP (트랜스진 1로서), EGFP (트랜스진 2로서) 및 mCherry (트랜스진 3으로서)를 N-, M- 및 C-Intres 게이트웨이 목적지 벡터로 재조합함으로써 이들 벡터의 3개 세트를 각각 시험하였으며, 이를 사용하여 U2OS 세포를 형질도입한 후, 히그로마이신 선택 또는 비-선택 배지에서 분할하고 배양하였다 (도 10b). 선택 후 2주에, 세포를 유동 세포계측법에 의해 분석하였다. 3-마커트론 히그로마이신 Intres 플라스미드의 3개 세트 모두는 비-선택된 배양물에서 <25%와 비교하여 >99%의 삼중 트랜스제닉 세포 선택을 지지하였다 (도 10c).

[0442] 본 발명자들은 4-마커트론 히그로마이신 Intres 유전자의 타당성을 추가로 시험하였다 (도 11). 여기서, 본 발명자들은 SspDnaB 인테인과 조합하여 류신 지퍼 모티프 11과 융합된 NpuDnaGEP 10으로 공지된 NpuDnaE 인테인의 향상된 변이체를 사용하였다. 구성요소 마커트론을 함유하는 모든 4개의 플라스미드의 형질도입은 히그로마이신 선택에서 생존한 세포를 생성한 반면, 리브-원-아웃 형질도입은 어떠한 생존도 생성하지 않았다 (표 2).

표 2. 지시된 플라스미드로부터 제조된 렌티바이러스로 ("+") 또는 렌티바이러스 없이 ("-") 형질도입된 세포의 생존

	플라스미드 115	플라스미드 116	플라스미드 117	플라스미드 118	생존
샘플 1	+	+	+	+	예
샘플 2	-	+	+	+	아니오
샘플 3	+	-	+	+	아니오
샘플 4	+	+	-	+	아니오
샘플 5	+	+	+	-	아니오

[0443]

[0444] 실시예 5. AAVS1 유전자좌에서의 이대립유전자 녹-인

[0445] CRISPR/Cas는 최근에 게놈 조작 및 편집을 위한 강력한 기술로 부상하였다. NHEJ-매개 삽입/결실 (indel)에 기초한 유전자 녹아웃(knockout)은 높은 빈도로 발생하지만, 외인성 복구 주형 (표적화 구축물이라고도 공지됨)을 사용하는 상동성 지정 복구(homology directed repair)(HDR)에 기초한 정확한 편집 및 녹-인은 비효율적이다. 본 발명자들은 분할 선택가능한 마커가 AAVS1 유전자좌에서 이대립유전자 녹-인을 갖는 세포를 풍부하게 하는데 사용될 수 있는지를 시험하였다. 본 발명자들은 표적 부위를 플랭킹하는 상동성 아암을 갖는 표적화 구축물을 구축하고, 숙주 유전자 PPP1R12C 중 하나인 인트론 내에 마커트론을 포획하기 위해 수용자-2A 펩티드를 스플라이싱하였다. 그러나, 본 발명자들은 이들 표적화 구축물을 사용하는 CRISPR/Cas 녹-인 실험 및 항생제 선택의 2주 후에 어떠한 살아있는 세포도 얻지 못하였다 (데이터는 나타내지 않음). 본 발명자들은 숙주 유전자 PPP1R12C의 내인성 프로모터가 항생제의 작용에 대항하기에 충분한 항생제 내성 단백질을 재구성하기 위해 마커트론의 충분한 발현을 구동할 수 없을 것으로 의심하였다. 그러므로, 본 발명자들은 활성이 독시사이클린 (dox) 농도에 의해 적정될 수 있는 TetO 프로모터에 의해 Intres 마커트론을 발현하는 대안적인 전략을 시험하였다. Intres-매개 이대립유전자 선택 대 전장 (FL) 비-분할 선택가능한 마커의 비교를 허용하기 위해, 본 발명자들은 여러 상이한 표적화 구축물 설계를 구현하였다. 먼저, 본 발명자들은 구성적(constitutive) EF1a 프로모터 하에 rtTA 및 dox-유도성 TetO 프로모터 하에 별도의 시험 Intres (예를 들어, Blast Intres)와 함께 전장 (FL) 내성 유전자 (예를 들어, Hygro)의 발현을 구동하였다 (도 12b, 플라스미드 109 및 110). 이는 동일한 구축물 내에 전장 및 분할 선택가능한 마커의 비교를 허용하였다. 동일한 TetO 프로모터에 의해 구동된 전장 대 분할 마커의 공정한 비교를 허용하기 위해, 본 발명자들은 2개의 유사한 플라스미드 107 및 108 (cf. 플라스미드 109 및 110)을 구축하였으며, 여기서 전장 항생제 내성 유전자 (Blast)는 TetO 프로모터의 하류에 위

치하였다 (도 12a). 이대립유전자 표적화의 단일-세포 정량화를 가능하게 하고, 2개의 트랜스진의 2개의 AAVS1 대립유전자로의 혼입의 타당성을 입증하기 위해, 본 발명자들은 자기-절단 2A 펩티드를 통해 시험 분할 또는 비-분할 마커의 하류에 EGFP 및 mScarlet 형광 유전자를 첨부하였다. 유사하게, Hygro Intres를 시험하기 위해, 본 발명자들은 FL Hygro 또는 Hygro Intres는 TetO의 하류에 위치하고 FL Blast는 EF1a의 하류에 위치하도록 EF1a 및 TetO-구동된 마커를 교체하였다 (도 12c-12d; 플라스미드 111-114). 본 발명자들은 Cas9 및 sgRNA 표적화 AAVS1을 함유하는 pX330-AAVS1 (플라스미드 106), 및 표적화 구축물의 상이한 쌍을 HEK293T 세포로 공동-형질감염하고, 이를 후속 계대배양에서 항생제를 갖지 않는, 블라스티시딘을 갖는 또는 히그로마이신을 갖는 삼중 독시사이클린-함유 배지로 분할하였다. 선택 후 2주에, 본 발명자들은 GFP 및 RFP 형광의 유동 세포계측법 측정에 의해 이대립유전자 표적화에 대해 배양물을 분석하였다 (도 12e). 예상된 바와 같이, 비-선택된 배양물은 작은 분율 (<1%)의 이대립유전자 녹-인 GFP+/RFP+ 세포를 보유하였다 (도 12e; 선택 = 없음). 상응하는 FL 항생제 내성 유전자가 표적화 구축물 상에 존재하는 항생제의 선택은 < 30% 이대립유전자 녹-인 세포를 생성하였다 (도 12e; Blast: TC a,c,d; Hygro: TC a,b,c). 대조적으로, 상응하는 Intres가 표적화 구축물 상에 존재하는 항생제의 선택은 75% (도 6e; Blast Intres: TC b) 및 88% (도 6e; Hygro Intres: TC d) 이대립유전자 녹-인 세포를 생성하였다

[0446] 상기 실시예에서, 본 발명자들은 2개 이상의 "비연결된" 트랜스진에 대한 선택을 허용할 수 있는 분할 항생제 내성 및 형광 단백질 유전자를 조작하였다. 선택가능한 마커에 비자연 잔기를 삽입함으로써, 본 발명자들은 새로운 고효율 분할점을 사용하여 조작에 이용가능한 위치를 확장할 수 있음을 보여주었다. 본 발명자들은 분할 선택가능한 마커가 CRISPR/Cas9 게놈 편집 실험에서 렌티바이러스 벡터 또는 유전자 표적화 구축물로 혼입되어, 이중 트랜스제네시스 또는 이대립유전자 녹-인을 갖는 세포의 풍부를 가능하게 할 수 있다는 것을 입증하였다. 2개 이상의 분할점을 조합함으로써, 본 발명자들은 3- 및 4-분할 마커를 생성하여 고도의 트랜스제닉 선택을 가능하게 할 수 있음을 보여주었다. 훨씬 고도의 분할 선택가능한 마커의 미래 개발은 수십 개의 트랜스진 또는 표적화된 녹-인을 함유하는 세포의 "과조작(hyper-engineering)"을 가능하게 할 수 있다.

[0447] 재료 및 방법

[0448] 클로닝

[0449] 각각의 마커트론을 위한 시험 플라스미드를 생성하기 위해, 본 발명자들은 먼저 그의 ORF를 함유하는 게이트웨이 공여자 플라스미드를 생성한 후, TagBFP2 (플라스미드 94: pLX-DEST-IRES-TagBFP2), EGFP (플라스미드 95: pLX-DEST-IRES-EGFP), 또는 mCherry (플라스미드 96: pLX-DEST-IRES-mCherry) 리포터를 갖는 렌티바이러스 목적지 벡터로 재조합하였으며, 이들은 퓨로마이신 내성 유전자를 제거하고 게이트웨이 카세트의 하류에 IRES-형광 유전자를 삽입함으로써 pLX302 (addgene.org/25896/)로부터 유래된 것이었다. 마커트론-ORF 게이트웨이 공여자 플라스미드는 인테인을, 선택가능한 마커의 단편의 코딩 서열과 조합한 후 서열- 및 라이게이션-독립적 클로닝(sequence- and ligation-independent cloning)(SLIC)에 의해 pCR8-GW-TOPO 플라스미드에 삽입하는 내포 융합 PCR 절차 (Li, M.Z. & Elledge, S.J. SLIC: a method for sequence-and ligation-independent cloning. Gene Synthesis: Methods and Protocols, 51-59 (2012))에 의해, 또는 선택가능한 마커의 관련 단편을 PCR-증폭시킨 후 SLIC에 의해 인테인 서열을 함유하는 "스캐폴드" 플라스미드 (플라스미드 27~32)에 삽입함으로써 생성하였다. 인테인을 코딩하는 DNA 서열을 호모 사피엔스에 대해 코돈 최적화하고, NpuDnaE 인테인을 코딩하는 AC1947GB, SspDnaB 인테인을 코딩하는 AC1949GB와 함께 GBlock (IDT)으로서 합성하였다. 이들 마커를 함유하는 플라스미드로부터 선택가능한 마커 단편을 증폭시켰다. 플라스미드에 대해서는 표 1을 참조한다.

[0450] 세포 배양

[0451] 모든 세포를 10% 소 태아 혈청 (FBS)(론자(Lonza)), 4% 글루타맥스(Glutamax) (깁코(Gibco)), 1% 피루브산나트륨 (깁코) 및 페니실린-스트렙토마이신 (깁코)을 갖는 둘베코 변형 이글 배지(Dulbecco's modified Eagle's medium)(DMEM) (시그마(Sigma))에서 배양하였다. 인큐베이터 조건은 37℃ 및 5% CO_2였다.

[0452] 바이러스 생성

[0453] pLP1, pLP2 및 VSV-G의 바이러스 패키징 믹스를, 리포펙타민(Lipofectamine) 3000을 사용하여 웰 당 $1.2x10^6$개 세포의 농도로 6-웰 플레이트에서 그 전날 접종된 Lenti-X 293T 세포 (클론테크(ClonTech))에 각각의 렌티바이러스 벡터와 함께 공동-형질감염시켰다. 형질감염 후 6시간에 배지를 교체한 다음, 밤새 인큐베이션하였다. 형질감염 후 28시간에 바이러스를 함유하는 배지 상등액을 45 uM PES 필터를 사용하여 여과한 후, 사용할 때까지 -80℃에서 저장하였다.

[0454] 형질도입

[0455] 형질도입 전날, 표적 세포 (HEK293T, MCF7, U2-OS)를 웰 당 $1.5x10^5$개 세포의 밀도로 12-웰 플레이트에 씨딩하였다. 형질도입 전에, 배지를 웰 당 1 mL의 10 μg/mL 폴리브렌을 함유하는 배지로 교체하였다. 250 μL의 각각의 바이러스 (2종의 바이러스가 첨가된 실험 샘플의 경우 총 500 μL)를 각각의 웰에 첨가하고 밤새 인큐베이션하였다. 감염 후 24시간에 배지를 교체하였다. 감염 후 4일에 세포를 이중 플레이트로 분할하였다. 감염 후 5일에 항생제 (히그로마이신)를 갖는 배지를 하나의 복제 플레이트의 웰 각각에 첨가하였다 (다른 하나는 선택 없이 유지됨). 항생제 선택은 FACS에 의한 분석전 2주 동안 계속하였다.

[0456] 형광-활성화 세포 분류법

[0457] 세포를 트립신으로 처리하고, 배지에 현탁시킨 후, HP Z230 워크스테이션에서 FACSDiVa 소프트웨어, 버전 8을 사용하여 LSRFortessa X-20 (비디 바이오사이언스(BD Bioscience)) 유동 세포계측기에서 분석하였다. 각각의 실행에서 5만건의 사건을 수집하였다.

[0458] <구축물 및 서열>

NpuDnaE(N)
CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDRGEQEVFEYCLEDGSLIRATKDHKF MTVDGQMLPIDEIFERELDLMRVDNLPN (SEQ ID NO: 7)

NpuDnaE(C)
IKIATRKYLGKQNVYDIGVERDHNFALKNGFIASN (SEQ ID NO: 8)

SspDnaB(N-S0)
CISGDSLISLASTGKRVSIKDLLDEKDFEIWAINEQTMKLESAKVSRVFCTGKKLVYILKTRLGRTIKATANHRF LTIDGWKRLDELSLKEHIALPRKLESSSLQL (SEQ ID NO: 9)

SspDnaB(C-S0)
SPEIEKLSQSDIYWDSIVSITETGVEEVFDLTVPGPHNFVANDIIVHN (SEQ ID NO: 10)

NpuDnaE(N)-LZA
CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDRGEQEVFEYCLEDGSLIRATKDHKF MTVDGQMLPIDEIFERELDLMRVDNLPNGGGGSGSAQLEKELQALEKKLAQLEWENQALEKELAQ (SEQ ID NO: 11)

LZB-NpuDnaGEP(C)
AQLKKKLQANKKELAQLKWKLQALKKKLAQGGGGSGSMIKIATRKYLGKQNVYDIGVGEPHNFALKNGFIA SN (SEQ ID NO: 12)

NpuDnaGFP(C)
IKIATRKYLGKQNVYDIGVGEPHNFALKNGFIASN (SEQ ID NO: 13)

LZA
AQLEKELQALEKKLAQLEWENQALEKELAQ (SEQ ID NO: 14)

LZB
AQLKKKLQANKKELAQLKWKLQALKKKLAQ (SEQ ID NO: 15)

[0459]

SspDnaE(N)

CLSFGTEILTVEYGPLPIGKIVSEEINCSVYSVDPEGRVYTQAIAQWHDRGEQEVLEYELEDGSVIRATSDHRFL TTDYQLLAIEEIFARQLDLLTLENIKQTEEALDNHRLPFPLLDAGTIK (SEQ ID NO:16)

SspDnaE(C)

VKVIGRRSLGVQRIFDIGLPQDHNFLLANGAIAAN (SEQ ID NO:17)

SspDnaB(N)

CISGDSLISLA (SEQ ID NO:18)

SspDnaB(C)

STGKRVSIKDLLDEKDFEIWAINEQTMKLESAKVSRVFCTGKKLVYILKTRLGRTIKATANHRFLTIDGWKRLD ELSLKEHIALPRKLESSSLQLSPEIEKLSQSDIYWDSIVSITETGVEEVFDLTVPGPHNFVANDIIVHN (SEQ ID NO:19)

[0460]

[0461] 플라스미드 3: pLX-Hygro(1-89)-NpuDnaE(N)-IRES-TagBFP2

[0462] 단백질 = Hygro(1-89)-NpuDnaE(N)

[0463] 벡터 서열 (서열식별번호: 20)

[0464] 아미노산 서열 (서열식별번호: 21)

[0465] 플라스미드 4: pLX-NpuDnaE(C)-Hygro(90-341)-IRES-mCherry

[0466] 단백질 = NpuDnaE(C)-Hygro(90-341)

[0467] 벡터 서열 (서열식별번호: 22)

[0468] 아미노산 서열 (서열식별번호: 23)

[0469] 플라스미드 5: pLX-Hygro(1-200)-SspDnaB(N)-IRES-TagBFP2

[0470] 단백질 = Hygro(1-200)-SspDnaB(N)

[0471] 벡터 서열 (서열식별번호: 24)

[0472] 아미노산 서열 (서열식별번호: 25)

[0473] 플라스미드 6: pLX-SspDnaB(C)-Hygro(201-341)-IRES-mCherry

[0474] 단백질 = SspDnaB(C)-Hygro(201-341)

[0475] 벡터 서열 (서열식별번호: 26)

[0476] 아미노산 서열 (서열식별번호: 27)

[0477] 플라스미드 7: pLX-Hygro(1-52)-NpuDnaE(N)-IRES-TagBFP2

[0478] 단백질 = Hygro(1-52)-NpuDnaE(N)

[0479] 벡터 서열 (서열식별번호: 28)

[0480] 아미노산 서열 (서열식별번호: 29)

[0481] 플라스미드 8: pLX-NpuDnaE(C)-Hygro(53-341)-IRES-mCherry

[0482] 단백질 = NpuDnaE(C)-Hygro(53-341)

[0483] 벡터 서열 (서열식별번호: 30)

[0484] 아미노산 서열 (서열식별번호: 31)

[0485] 플라스미드 9: pLX-Hygro(1-240)-NpuDnaE(N)-IRES-TagBFP2

[0486] 단백질 = Hygro(1-240)-NpuDnaE(N)

[0487] 벡터 서열 (서열식별번호: 32)

[0488] 아미노산 서열 (서열식별번호: 33)

[0489] 플라스미드 10: pLX-NpuDnaE(C)-Hygro(241-341)-IRES-mCherry

[0490] 단백질 = NpuDnaE(C)-Hygro(241-341)

[0491] 벡터 서열 (서열식별번호: 34)

[0492] 아미노산 서열 (서열식별번호: 35)

[0493] 플라스미드 11: pLX-Hygro(1-292)-NpuDnaE(N)-IRES-TagBFP2

[0494] 단백질 = Hygro(1-292)-NpuDnaE(N)

[0495] 벡터 서열 (서열식별번호: 36)

[0496] 아미노산 서열 (서열식별번호: 37)

[0497] 플라스미드 12: pLX-NpuDnaE(C)-Hygro(293-341)-IRES-mCherry

[0498] 단백질 = NpuDnaE(C)-Hygro(293-341)

[0499] 벡터 서열 (서열식별번호: 38)

[0500] 아미노산 서열 (서열식별번호: 39)

[0501] 플라스미드 13: pLX-Blast(1-102)-NpuDnaE(N)-IRES-TagBFP2

[0502] 단백질 = Blast(1-102)-NpuDnaE(N)

[0503] 벡터 서열 (서열식별번호: 40)

[0504] 아미노산 서열 (서열식별번호: 41)

[0505] 플라스미드 14: pLX-NpuDnaE(C)-Blast(103-140)-IRES-mCherry

[0506] 단백질 = NpuDnaE(C)-Blast(103-140)

[0507] 벡터 서열 (서열식별번호: 42)

[0508] 아미노산 서열 (서열식별번호: 43)

[0509] 플라스미드 17: pLX-Puro(1-119)-NpuDnaE(N)-IRES-TagBFP2

[0510] 단백질 = Puro(1-119)-NpuDnaE(N)

[0511] 벡터 서열 (서열식별번호: 44)

[0512] 아미노산 서열 (서열식별번호: 45)

[0513] 플라스미드 18: pLX-NpuDnaE(C)-Puro(insCys;120-199)-IRES-mCherry

[0514] 단백질 = NpuDnaE(C)-Puro(insCys;120-199)

[0515] 벡터 서열 (서열식별번호: 46)

[0516] 아미노산 서열 (서열식별번호: 47)

[0517] 플라스미드 19: pLX-Puro(1-100)-SspDnaB(N-S0)-IRES-TagBFP2

[0518] 단백질 = Puro(1-100)-SspDnaB(N-S0)

[0519] 벡터 서열 (서열식별번호: 48)

[0520] 아미노산 서열 (서열식별번호: 49)

[0521] 플라스미드 20: pLX-SspDnaB(C-S0)-Puro(101-199)-IRES-mCherry

[0522] 단백질 = SspDnaB(C-S0)-Puro(101-199)

[0523] 벡터 서열 (서열식별번호: 50)

[0524] 아미노산 서열 (서열식별번호: 51)

[0525] 플라스미드 21: pLX-Neo(1-133)-NpuDnaE(N)-IRES-TagBFP2

[0526] 단백질 = Neo(1-133)-NpuDnaE(N)

[0527] 벡터 서열 (서열식별번호: 52)

[0528] 아미노산 서열 (서열식별번호: 53)

[0529] 플라스미드 22: pLX-NpuDnaE(C)-Neo(134-267)-IRES-mCherry

[0530] 단백질 = NpuDnaE(C)-Neo(134-267)

[0531] 벡터 서열 (서열식별번호: 54)

[0532] 아미노산 서열 (서열식별번호: 55)

[0533] 플라스미드 23: pLX-Neo(1-194)-NpuDnaE(N)-IRES-TagBFP2

[0534] 단백질 = Neo(1-194)-NpuDnaE(N)

[0535] 벡터 서열 (서열식별번호: 56)

[0536] 아미노산 서열 (서열식별번호: 57)

[0537] 플라스미드 24: pLX-NpuDnaE(C)-Neo(195-267)-IRES-mCherry

[0538] 단백질 = NpuDnaE(C)-Neo(195-267)

[0539] 벡터 서열 (서열식별번호: 58)

[0540] 아미노산 서열 (서열식별번호: 59)

[0541] 플라스미드 25: pLX-NpuDnaE(C)_Hygro(53-89)-NpuDnaE(N)-IRES-GFP

[0542] 단백질 = NpuDnaE(C)_Hygro(53-89)-NpuDnaE(N)

[0543] 벡터 서열 (서열식별번호: 60)

[0544] 아미노산 서열 (서열식별번호: 61)

[0545] 플라스미드 26: pLX-NpuDnaE(C)_Hygro(53-239)-NpuDnaE(N)-IRES-GFP

[0546] 단백질 = NpuDnaE(C)_Hygro(53-239)-NpuDnaE(N)

[0547] 벡터 서열 (서열식별번호: 62)

[0548] 아미노산 서열 (서열식별번호: 63)

[0549] 플라스미드 27: pCR8-BsaI->ccdbCam<-BsaI-NpuDnaE(N)-MD1-68-15 (서열식별번호: 64)

[0550] 플라스미드 28: pCR8-NpuDnaE(C)_BsaI->ccdbCam<-BsaI-MD1-68-18 (서열식별번호: 65)

[0551] 플라스미드 29: pCR8-BsaI->ccdbCam<-BsaI-SspDnaE(N)-MD1-68-12 (서열식별번호: 66)

[0552] 플라스미드 30: pCR8-SspDnaE(C)_BsaI->ccdbCam<-BsaI-MD1-68-13 (서열식별번호: 67)

[0553] 플라스미드 31: pCR8-BsaI->ccdbCam<-BsaI-SspDnaB(N-S0)-25-135-18 (서열식별번호: 68)

[0554] 플라스미드 32: pCR8-SspDnaB(C-S0)_BsaI->ccdbCam<-BsaI-25-155-41 (서열식별번호: 69)

[0555] 플라스미드 33: pLX-mScarlet(1-46)-NpuDnaE(N)_LZA-IRES-TagBFP2

[0556] 단백질 = mScarlet(1-46)-NpuDnaE(N)_LZA

[0557] 벡터 서열 (서열식별번호: 70)

[0558] 아미노산 서열 (서열식별번호: 71)

[0559] 플라스미드 34: pLX-LZB_NpuDnaE(C)-mScarlet(insCys;47-232)-IRES-TagBFP2

[0560] 단백질 = LZB_NpuDnaE(C)-mScarlet(insCys;47-232)

[0561] 벡터 서열 (서열식별번호: 72)

[0562] 아미노산 서열 (서열식별번호: 73)

[0563] 플라스미드 35: pLX-mScarlet(1-48)-NpuDnaE(N)_LZA-IRES-TagBFP2

[0564] 단백질 = mScarlet(1-48)-NpuDnaE(N)_LZA

[0565] 벡터 서열 (서열식별번호: 74)

[0566] 아미노산 서열 (서열식별번호: 75)

[0567] 플라스미드 36: pLX-LZB_NpuDnaE(C)-mScarlet(insCys;49-232)-IRES-GFP

[0568] 단백질 = LZB_NpuDnaE(C)-mScarlet(insCys;49-232)

[0569] 벡터 서열 (서열식별번호: 76)

[0570] 아미노산 서열 (서열식별번호: 77)

[0571] 플라스미드 37: pLX-mScarlet(1-51)-NpuDnaE(N)_LZA -IRES-TagBFP2

[0572] 단백질 = mScarlet(1-51)-NpuDnaE(N)_LZA

[0573] 벡터 서열 (서열식별번호: 78)

[0574] 아미노산 서열 (서열식별번호: 79)

[0575] 플라스미드 38: pLX-LZB_NpuDnaE(C)-mScarlet(insCys;52-232)-IRES-GFP

[0576] 단백질 = LZB_NpuDnaE(C)-mScarlet(insCys;52-232)

[0577] 벡터 서열 (서열식별번호: 80)

[0578] 아미노산 서열 (서열식별번호: 81)

[0579] 플라스미드 39: pLX-mScarlet(1-75)-NpuDnaE(N)_LZA-IRES-TagBFP2

[0580] 단백질 = mScarlet(1-75)-NpuDnaE(N)_LZA

[0581] 벡터 서열 (서열식별번호: 82)

[0582] 아미노산 서열 (서열식별번호: 83)

[0583] 플라스미드 40: pLX-LZB_NpuDnaE(C)-mScarlet(insCys;76-232)-IRES-GFP

[0584] 단백질 = LZB_NpuDnaE(C)-mScarlet(insCys;76-232)

[0585] 벡터 서열 (서열식별번호: 84)

[0586] 아미노산 서열 (서열식별번호: 85)

[0587] 플라스미드 41: pLX-mScarlet(1-122)-NpuDnaE(N)_LZA-IRES-TagBFP2

[0588] 단백질 = mScarlet(1-122)-NpuDnaE(N)_LZA

[0589] 벡터 서열 (서열식별번호: 86)

[0590] 아미노산 서열 (서열식별번호: 87)

[0591] 플라스미드 42: pLX-LZB_NpuDnaE(C)-mScarlet(insCys;123-232)-IRES-GFP

[0592] 단백질 = LZB_NpuDnaE(C)-mScarlet(insCys;123-232)

[0593] 벡터 서열 (서열식별번호: 88)

[0594] 아미노산 서열 (서열식별번호: 89)

[0595] 플라스미드 43: pLX-mScarlet(1-140)-NpuDnaE(N)_LZA-IRES-TagBFP2

[0596] 단백질 = mScarlet(1-140)-NpuDnaE(N)_LZA

[0597] 벡터 서열 (서열식별번호: 90)

[0598] 아미노산 서열 (서열식별번호: 91)

[0599] 플라스미드 44: pLX-LZB_NpuDnaE(C)-mScarlet(insCys;141-232)-IRES-GFP

[0600] 단백질 = LZB_NpuDnaE(C)-mScarlet(insCys;141-232)

[0601] 벡터 서열 (서열식별번호: 92)

[0602] 아미노산 서열 (서열식별번호: 93)

[0603] 플라스미드 45: pLX-mScarlet(1-163)-NpuDnaE(N)_LZA-IRES-TagBFP2

[0604] 단백질 = mScarlet(1-163)-NpuDnaE(N)_LZA

[0605] 벡터 서열 (서열식별번호: 94)

[0606] 아미노산 서열 (서열식별번호: 95)

[0607] 플라스미드 46: pLX-LZB_NpuDnaE(C)-mScarlet(insCys;164-232)-IRES-GFP

[0608] 단백질 = LZB_NpuDnaE(C)-mScarlet(insCys;164-232)

[0609] 벡터 서열 (서열식별번호: 96)

[0610] 아미노산 서열 (서열식별번호: 97)

[0611] 플라스미드 47: pCR8-TagBFP2

[0612] 단백질 = TagBFP2

[0613] 벡터 서열 (서열식별번호: 98)

[0614] 아미노산 서열 (서열식별번호: 99)

[0615] 플라스미드 48: pCR8-mCherry

[0616] 단백질 = mCherry

[0617] 벡터 서열 (서열식별번호: 100)

[0618] 아미노산 서열 (서열식별번호: 101)

[0619] 플라스미드 49: pLX-DEST-IRES-Hygro(1-89)-NpuDnaE(N)

[0620] 단백질 = Hygro(1-89)-NpuDnaE(N)

[0621] 벡터 서열 (서열식별번호: 102)

[0622] 아미노산 서열 (서열식별번호: 103)

[0623] 플라스미드 50: pLX-DEST-IRES-NpuDnaE(C)-Hygro(90-341)

[0624] 단백질 = NpuDnaE(C)-Hygro(90-341)

[0625] 벡터 서열 (서열식별번호: 104)

[0626] 아미노산 서열 (서열식별번호: 105)

[0627] 플라스미드 51: pLX-[TagBFP2]-IRES-Hygro(1-89)-NpuDnaE(N)

[0628] 벡터 서열 (서열식별번호: 106)

[0629] 플라스미드 52: pLX-[mCherry]-IRES-NpuDnaE(C)-Hygro(90-341)

[0630] 벡터 서열 (서열식별번호: 107)

[0631] 플라스미드 53: pLX-DEST-IRES-Puro(1-119)-NpuDnaE(N)

[0632] 단백질 = Puro(1-119)-NpuDnaE(N)

[0633] 벡터 서열 (서열식별번호: 108)

[0634] 아미노산 서열 (서열식별번호: 109)

[0635] 플라스미드 54: pLX-DEST-IRES-NpuDnaE(C)-Puro(120-199)

[0636] 단백질 = NpuDnaE(C)-Puro(120-199)

[0637] 벡터 서열 (서열식별번호: 110)

[0638] 아미노산 서열 (서열식별번호: 111)

[0639] 플라스미드 55: pLX-[TagBFP2]-IRES-Puro(1-119)-NpuDnaE(N)

[0640] 벡터 서열 (서열식별번호: 112)

[0641] 플라스미드 56: pLX-[mCherry]-IRES-NpuDnaE(C)-Puro(120-199)

[0642] 벡터 서열 (서열식별번호: 113)

[0643] 플라스미드 57: pLX-DEST-IRES-Neo(1-194)-NpuDnaE(N)

[0644] 단백질 = Neo(1-194)-NpuDnaE(N)

[0645] 벡터 서열 (서열식별번호: 114)

[0646] 아미노산 서열 (서열식별번호: 115)

[0647] 플라스미드 58: pLX-DEST-IRES-NpuDnaE(C)-Neo(195-267)

[0648] 단백질 = NpuDnaE(C)-Neo(195-267)

[0649] 벡터 서열 (서열식별번호: 116)

[0650] 아미노산 서열 (서열식별번호: 117)

[0651] 플라스미드 59: pLX-[TagBFP2]-IRES-Neo(1-194)-NpuDnaE(N)

[0652] 벡터 서열 (서열식별번호: 118)

[0653] 플라스미드 60: pLX-[mCherry]-IRES-NpuDnaE(C)-Neo(195-267)

[0654] 벡터 서열 (서열식별번호: 119)

[0655] 플라스미드 61: pLX-mScarlet(1-51)-NpuDnaE(N)-LZA-IRES-TagBFP2

[0656] 단백질 = mScarlet(1-51)-NpuDnaE(N)-LZA

[0657] 벡터 서열 (서열식별번호: 120)

[0658] 아미노산 서열 (서열식별번호: 121)

[0659] 플라스미드 62: pLX-LZB-NpuDnaE(C)-mScarlet(^C,52-163)-NpuDnaE(N)_LZA-IRES-EGFP

[0660] 단백질 = LZB-NpuDnaE(C)-mScarlet(^C;52-163)-NpuDnaE(N)_LZA

[0661] 벡터 서열 (서열식별번호: 122)

[0662] 아미노산 서열 (서열식별번호: 123)

[0663] 플라스미드 63: pLX-LZB-NpuDnaE(C)-mScarlet(^C;164-232)-IRES-EGFP

[0664] 단백질 = LZB-NpuDnaE(C)-mScarlet(^C;164-232)

[0665] 벡터 서열 (서열식별번호: 124)

[0666] 아미노산 서열 (서열식별번호: 125)

[0667] 플라스미드 64: pLX-Hygro(1-69)-NpuDnaE(N)-IRES-TagBFP2

[0668] 단백질 = Hygro(1-69)-NpuDnaE(N)

[0669] 벡터 서열 (서열식별번호: 126)

[0670] 아미노산 서열 (서열식별번호: 127)

[0671] 플라스미드 65: pLX-NpuDnaE(C)-Hygro(^C;70-341)-IRES-mCherry

[0672] 단백질 = NpuDnaE(C)-Hygro(^C;70-341)

[0673] 벡터 서열 (서열식별번호: 128)

[0674] 아미노산 서열 (서열식별번호: 129)

[0675] 플라스미드 66: pLX-Hygro(1-131)-NpuDnaE(N)-IRES-TagBFP2

[0676] 단백질 = Hygro(1-131)-NpuDnaE(N)

[0677] 벡터 서열 (서열식별번호: 130)

[0678] 아미노산 서열 (서열식별번호: 131)

[0679] 플라스미드 67: pLX-NpuDnaE(C)-Hygro(^C;132-341)-IRES-mCherry

[0680] 단백질 = NpuDnaE(C)-Hygro(^C;132-341)

[0681] 벡터 서열 (서열식별번호: 132)

[0682] 아미노산 서열 (서열식별번호: 133)

[0683] 플라스미드 68: pLX-Hygro(1-171)-NpuDnaE(N)-IRES-TagBFP2

[0684] 단백질 = Hygro(1-171)-NpuDnaE(N)

[0685] 벡터 서열 (서열식별번호: 134)

[0686] 아미노산 서열 (서열식별번호: 135)

[0687] 플라스미드 69: pLX-NpuDnaE(C)-Hygro(^C;172-341)-IRES-mCherry

[0688] 단백질 = NpuDnaE(C)-Hygro(^C;172-341)

[0689] 벡터 서열 (서열식별번호: 136)

[0690] 아미노산 서열 (서열식별번호: 137)

[0691] 플라스미드 70: pLX-Hygro(1-218)-NpuDnaE(N)-IRES-TagBFP2

[0692] 단백질 = Hygro(1-218)-NpuDnaE(N)

[0693] 벡터 서열 (서열식별번호: 138)

[0694] 아미노산 서열 (서열식별번호: 139)

[0695] 플라스미드 71: pLX-NpuDnaE(C)-Hygro(^C;219-341)-IRES-mCherry

[0696] 단백질 = NpuDnaE(C)-Hygro(^C;219-341)

[0697] 벡터 서열 (서열식별번호: 140)

[0698] 아미노산 서열 (서열식별번호: 141)

[0699] 플라스미드 72: pLX-Hygro(1-259)-NpuDnaE(N)-IRES-TagBFP2

[0700] 단백질 = Hygro(1-259)-NpuDnaE(N)

[0701] 벡터 서열 (서열식별번호: 142)

[0702] 아미노산 서열 (서열식별번호: 143)

[0703] 플라스미드 73: pLX-NpuDnaE(C)-Hygro(^C;260-341)-IRES-mCherry

[0704] 단백질 = NpuDnaE(C)-Hygro(^C;260-341)

[0705] 벡터 서열 (서열식별번호: 144)

[0706] 아미노산 서열 (서열식별번호: 145)

[0707] 플라스미드 74: pLX-Hygro(1-277)-NpuDnaE(N)-IRES-TagBFP2

[0708] 단백질 = Hygro(1-277)-NpuDnaE(N)

[0709] 벡터 서열 (서열식별번호: 146)

[0710] 아미노산 서열 (서열식별번호: 147)

[0711] 플라스미드 75: pLX-NpuDnaE(C)-Hygro(^C; 278-341)-IRES-mCherry

[0712] 단백질 = NpuDnaE(C)-Hygro(^C;278-341)

[0713] 벡터 서열 (서열식별번호: 148)

[0714] 아미노산 서열 (서열식별번호: 149)

[0715] 플라스미드 76: pLX-Puro(1-32)-NpuDnaE(N)-IRES-TagBFP2

[0716] 단백질 = Puro(1-32)-NpuDnaE(N)

[0717] 벡터 서열 (서열식별번호: 150)

[0718] 아미노산 서열 (서열식별번호: 151)

[0719] 플라스미드 77: pLX-NpuDnaE(C)-Puro(^C;33-199)-IRES-mCherry

[0720] 단백질 = NpuDnaE(C)-Puro(^C;33-199)

[0721] 벡터 서열 (서열식별번호: 152)

[0722] 아미노산 서열 (서열식별번호: 153)

[0723] 플라스미드 78: pLX-Puro(1-84)-NpuDnaE(N)-IRES-TagBFP2

[0724] 단백질 = Puro(1-84)-NpuDnaE(N)

[0725] 벡터 서열 (서열식별번호: 154)

[0726] 아미노산 서열 (서열식별번호: 155)

[0727] 플라스미드 79: pLX-NpuDnaE(C)-Puro(^C;85-199)-IRES-mCherry

[0728] 단백질 = NpuDnaE(C)-Puro(^C;85-199)

[0729] 벡터 서열 (서열식별번호: 156)

[0730] 아미노산 서열 (서열식별번호: 157)

[0731] 플라스미드 80: pLX-Puro(1-137)-NpuDnaE(N)-IRES-TagBFP2

[0732] 단백질 = Puro(1-137)-NpuDnaE(N)

[0733] 파일 = pLX-[PuroKC3(N)-NpuDnaE(N)-25-131-29"]-IRES-TagBFP2-25-133-6

[0734] 벡터 서열 (서열식별번호: 158)

[0735] 아미노산 서열 (서열식별번호: 159)

[0736] 플라스미드 81: pLX-NpuDnaE(C)-Puro(^C;138-199)-IRES-mCherry

[0737] 단백질 = NpuDnaE(C)-Puro(^C;138-199)

[0738] 벡터 서열 (서열식별번호: 160)

[0739] 아미노산 서열 (서열식별번호: 161)

[0740] 플라스미드 82: pLX-Puro(1-158)-NpuDnaE(N)-IRES-TagBFP2

[0741] 단백질 = Puro(1-158)-NpuDnaE(N)

[0742] 벡터 서열 (서열식별번호: 162)

[0743] 아미노산 서열 (서열식별번호: 163)

[0744] 플라스미드 83: pLX-NpuDnaE(C)-Puro(^C;159-199)-IRES-mCherry

[0745] 단백질 = NpuDnaE(C)-Puro(^C;159-199)

[0746] 벡터 서열 (서열식별번호: 164)

[0747] 아미노산 서열 (서열식별번호: 165)

[0748] 플라스미드 84: pLX-Puro(1-180)-NpuDnaE(N)-IRES-TagBFP2

[0749] 단백질 = Puro(1-180)-NpuDnaE(N)

[0750] 벡터 서열 (서열식별번호: 166)

[0751] 아미노산 서열 (서열식별번호: 167)

[0752] 플라스미드 85: pLX-NpuDnaE(C)-Puro(^C;181-199)-IRES-mCherry

[0753] 단백질 = NpuDnaE(C)-Puro(^C;181-199)

[0754] 벡터 서열 (서열식별번호: 168)

[0755] 아미노산 서열 (서열식별번호: 169)

[0756] 플라스미드 86: pLX-Blast(1-58)-NpuDnaE(N)-IRES-TagBFP2

[0757] 단백질 = Blast(1-58)-NpuDnaE(N)

[0758] 벡터 서열 (서열식별번호: 170)

[0759] 아미노산 서열 (서열식별번호: 171)

[0760] 플라스미드 87: pLX-NpuDnaE(C)-Blast(59-140)-IRES-mCherry

[0761] 단백질 = NpuDnaE(C)-Blast(59-140)

[0762] 벡터 서열 (서열식별번호: 172)

[0763] 아미노산 서열 (서열식별번호: 173)

[0764] 플라스미드 88: pLX-NpuDnaE(C)-HygroBA-SspDnaB(N-S0)-IRES-EGFP

[0765] 단백질 = NpuDnaE(C)-Hygro(53-200)-SspDnaB(N-S0)

[0766] 벡터 서열 (서열식별번호: 174)

[0767] 아미노산 서열 (서열식별번호: 175)

[0768] 플라스미드 89: pLX-SspDnaB(C-S0)-Hygro(201-341)-IRES-mCherry

[0769] 단백질 = SspDnaB(C-S0)-Hygro(201-341)

[0770] 벡터 서열 (서열식별번호: 176)

[0771] 아미노산 서열 (서열식별번호: 177)

[0772] 플라스미드 90: pLX-NpuDnaE(C)-Hygro(90-200)-SspDnaB(N-S0)-IRES-EGFP

[0773] 단백질 = NpuDnaE(C)-Hygro(90-200)-SspDnaB(N-S0)

[0774] 벡터 서열 (서열식별번호: 178)

[0775] 아미노산 서열 (서열식별번호: 179)

[0776] 플라스미드 91: pLX-Hygro(1-200)-SspDnaB(N-S0)-IRES-TagBFP2

[0777] 단백질 = Hygro(1-200)-SspDnaB(N-S0)

[0778] 벡터 서열 (서열식별번호: 180)

[0779] 아미노산 서열 (서열식별번호: 181)

[0780] 플라스미드 92: pLX-SspDnaB(C-S0)-Hygro(201-240)-NpuDnaE(N)-IRES-EGFP

[0781] 단백질 = SspDnaB(C-S0)-Hygro(201-240)-NpuDnaE(N)

[0782] 벡터 서열 (서열식별번호: 182)

[0783] 아미노산 서열 (서열식별번호: 183)

[0784] 플라스미드 93: pLX-SspDnaB(C-S0)-Hygro(201-292)-NpuDnaE(N)-IRES-EGFP

[0785] 단백질 = SspDnaB(C-S0)-Hygro(201-292)-NpuDnaE(N)

[0786] 벡터 서열 (서열식별번호: 184)

[0787] 아미노산 서열 (서열식별번호: 185)

[0788] 플라스미드 94: pLX-DEST-IRES-TagBFP2 (서열식별번호: 186)

[0789] 플라스미드 95: pLX-DEST-IRES-EGFP (서열식별번호: 187)

[0790] 플라스미드 96: pLX-DEST-IRES-mCherry (서열식별번호: 188)

[0791] 플라스미드 97: pLX-Hygro-IRES-TagBFP2

[0792] 벡터 서열 (서열식별번호: 189)

[0793] 플라스미드 98: pLX-Hygro-IRES-mCherry

[0794] 벡터 서열 (서열식별번호: 190)

[0795] 플라스미드 99: pLX-Puro-IRES-TagBFP2

[0796] 벡터 서열 (서열식별번호: 191)

[0797] 플라스미드 100: pLX-Puro-IRES-mCherry

[0798] 벡터 서열 (서열식별번호: 192)

[0799] 플라스미드 101: pLX-Hygro-IRES-EGFP

[0800] 벡터 서열 (서열식별번호: 193)

[0801] 플라스미드 102: pLX-NLS_GFP-IRES-Hygro

[0802] 벡터 서열 (서열식별번호: 194)

[0803] 플라스미드 103: pLX-LifeAct_mCherry-IRES-Hygro

[0804] 벡터 서열 (서열식별번호: 195)

[0805] 플라스미드 104: pLX-NLS_GFP-IRES-Hygro(1-89)-NpuDnaE(N)

[0806] 벡터 서열 (서열식별번호: 196)

[0807] 플라스미드 105: pLX-LifeAct_mScarlet-IRES- NpuDnaE(C)-Hygro(90-341)

[0808] 벡터 서열 (서열식별번호: 197)

[0809] 플라스미드 106: pX330-AAVS1

[0810] sgRNA 스페이서 서열: gACCCCACAGTGGGGCCACTA (첫 번째 g는 게놈과 매칭하지 않음) (서열식별번호: 198)

[0811] 벡터 서열 (서열식별번호: 199)

[0812] 플라스미드 107: pAAVS1-Nst-EF1aHygro2ArtTA3(-)_TetO-Blast-P2A-EGFP

[0813] 벡터 서열 (서열식별번호: 200)

[0814] 플라스미드 108: pAAVS1-Nst-EF1aHygro2ArtTA3(-)_TetO-Blast-P2A-mScarlet

[0815] 벡터 서열 (서열식별번호: 201)

[0816] 플라스미드 109: pAAVS1-Nst-EF1aHygro2ArtTA3(-)_TetO-Blast(1-102)_NpuDnaE(N)-P2A-EGFP

[0817] 벡터 서열 (서열식별번호: 202)

[0818] 플라스미드 110: pAAVS1-Nst-EF1aHygro2ArtTA3(-)_TetO-NpuDnaE(C)_Blast(103-140)-P2A-mScarlet

[0819] 벡터 서열 (서열식별번호: 203)

[0820] 플라스미드 111: pAAVS1-Nst-EF1aBlast2ArtTA3(-)_TetO-Hygro-P2A-NTR-E2A-EGFP

[0821] 벡터 서열 (서열식별번호: 204)

[0822] 플라스미드 112: pAAVS1-Nst-EF1aBlast2ArtTA3(-)_TetO-Hygro-P2A-NTR-E2A-mCherry

[0823] 벡터 서열 (서열식별번호: 205)

[0824] 플라스미드 113: pAAVS1-Nst-EF1aBlast2ArtTA3(-)_TetO- Hygro(1-89)-NpuDnaE(N)-P2A-NTR-E2A-EGFP

[0825] 벡터 서열 (서열식별번호: 206)

[0826] 플라스미드 114: pAAVS1-Nst-EF1aBlast2ArtTA3(-)_TetO- NpuDnaE(C)-Hygro(90-341)-P2A-NTR-E2A-mCherry

[0827] 벡터 서열 (서열식별번호: 207)

[0828] 플라스미드 115: pLX-Hygro(1-89)_NpuDnaE(N)_LZA-IRES-TagBFP2

[0829] 단백질 = Hygro(1-89)-NpuDnaE(N)-LZA

[0830] 벡터 서열 (서열식별번호: 208)

[0831] 아미노산 서열 (서열식별번호: 209)

[0832] 플라스미드 116: pLX-LZB_NpuDnaGEP(C)_Hygro(90-200)_SspDnaB(N-S0)-IRES-GFP

[0833] 단백질 = LZB-NpuDnaGEP(C)-Hygro(90-200)-SspDnaB(N-S0)

[0834] 벡터 서열 (서열식별번호: 210)

[0835] 아미노산 서열 (서열식별번호: 211)

[0836] 플라스미드 117: pLX-SspDnaB(C-S0)_Hygro(201-240)_NpuDnaE(N)_LZA-IRES-GFP

[0837] 단백질 = SspDnaB(C-S0)-Hygro(201-240)-NpuDnaE(N)-LZA

[0838] 벡터 서열 (서열식별번호: 212)

[0839] 아미노산 서열 (서열식별번호: 213)

[0840] 플라스미드 118: pLX-LZB_NpuDnaGEP(C)_Hygro(241-341)-IRES-mCherry

[0841] 단백질 = LZB-NpuDnaGEP(C)-Hygro(241-341)

[0842] 벡터 서열 (서열식별번호: 214)

[0843] 아미노산 서열 (서열식별번호: 215)

[0844] AC1947GB (서열식별번호: 216)

[0845] AC1949GB (서열식별번호: 217)

[0846] pCR8-ccdbCam (서열식별번호: 218)

[0847] 참고문헌

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[0848]

[0849] 본원에 개시된 모든 참고문헌, 특허 및 특허 출원은 각각이 인용되는 주제와 관련하여 참조로 포함되며, 일부 경우에 문헌의 전체를 포함할 수 있다.

[0850] 본원의 명세서 및 청구범위에서 사용된 단수 형태는 달리 명백하게 지시되지 않는 한, "적어도 하나"를 의미하는 것으로 이해되어야 한다.

[0851] 또한, 달리 명백하게 지시되지 않는 한, 하나 초과의 단계 또는 작용을 포함하는 본원에 청구된 임의의 방법에서, 방법의 단계 또는 작용의 순서는 방법의 단계 또는 작용이 인용된 순서로 반드시 제한되는 것은 아니라는 것이 이해되어야 한다.

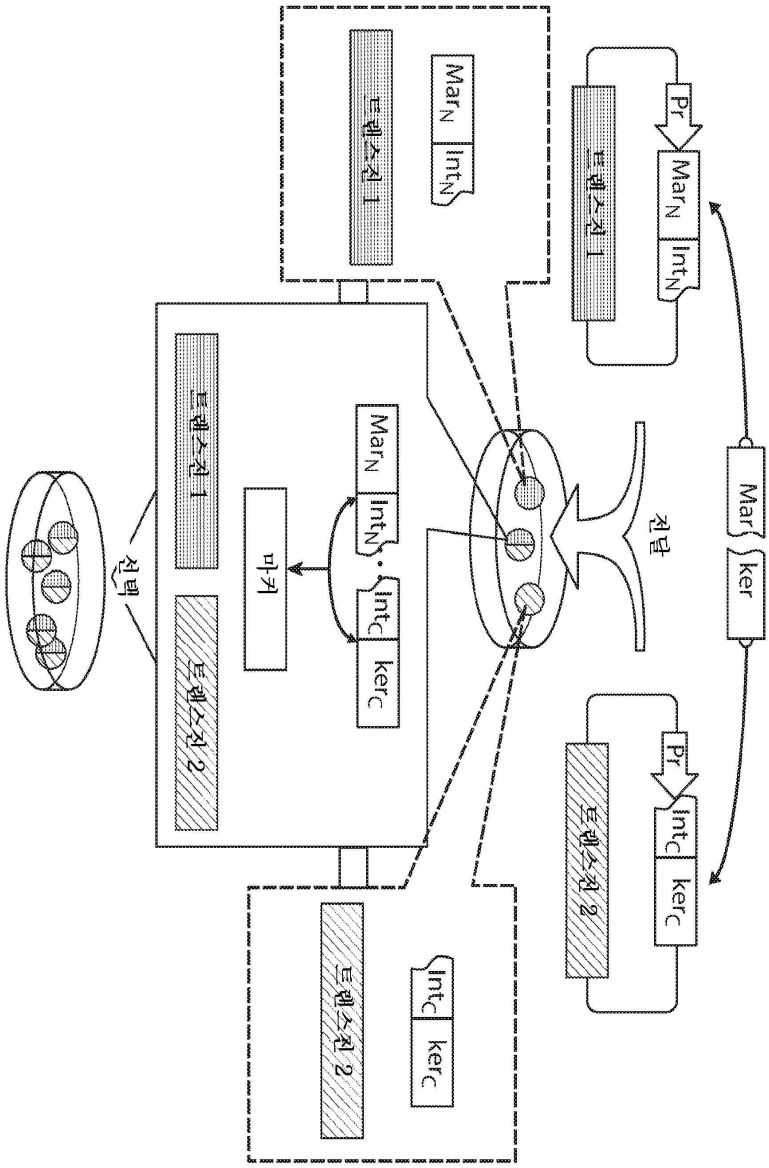
[0852] 상기 명세서 뿐만 아니라 청구범위에서, 모든 연결구, 예컨대 "포함하는(comprising)", "포함하는(including)", "보유하는" "갖는", "함유하는", "수반하는", "유지하는", "로 구성되는(composed of)" 등은 개방형, 즉 포함하나 이에 제한되지는 않는 것을 의미하는 것으로 이해되어야 한다. 연결구 "로 이루어진" 및 "로 본질적으로 이루어진"만이 미국 특허청 특허 심사 절차 매뉴얼, 섹션 2111.03에 기술된 바와 같이 각각 폐쇄형 또는 반-폐쇄형 연결구이다.

[0853] 수치 앞의 용어 "약" 및 "실질적으로"는 인용된 수치의 ±10%를 의미한다.

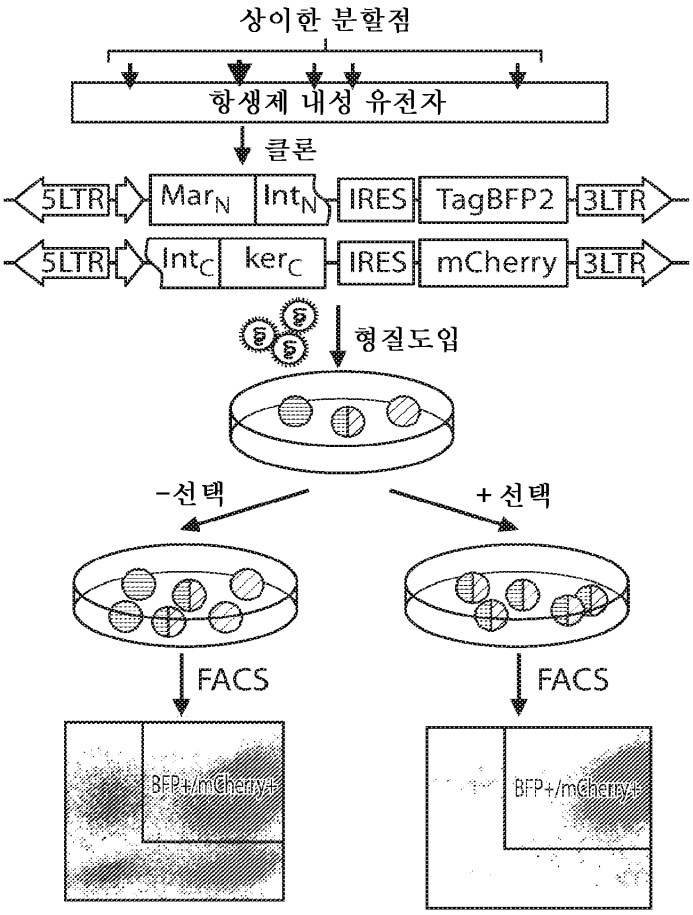
[0854] 값의 범위가 제공되는 경우, 범위의 상단 및 하단 사이의 각각의 값은 본원에서 구체적으로 고려되고 설명된다.

도면

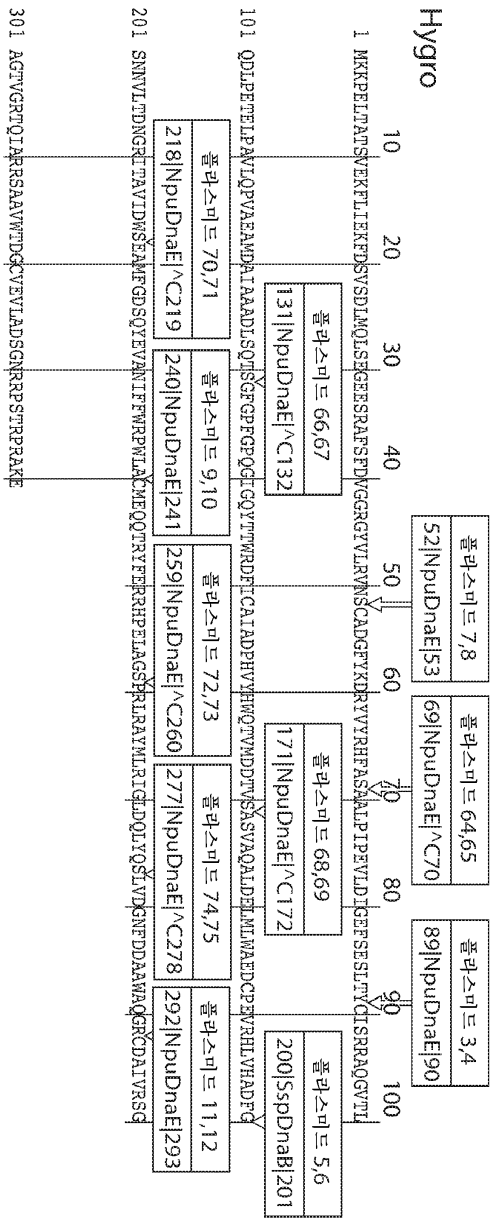
도면1a


트랜스진 1
Mar_N
Int_N
Pr
Mar_N
Int_N
트랜스진 1
Mar
ker
전달
Mar_N
Int_N
Int_C
ker_C
마커
선택
트랜스진 1
트랜스진 2
Pr
Int_C
ker_C
트랜스진 2
Int_C
ker_C
트랜스진 2

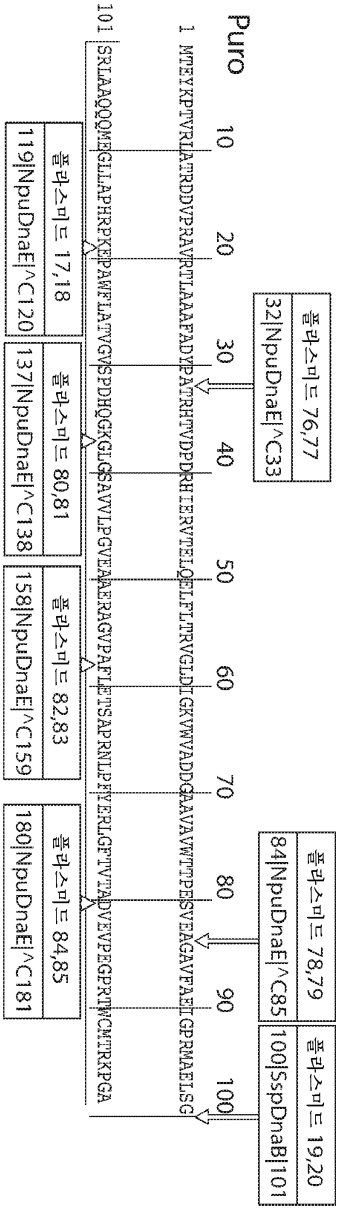
도면1b


상이한 분할점
항생제 내성 유전자
클론
5LTR
Mar$_N$
Int$_N$
IRES
TagBFP2
3LTR
5LTR
Int$_C$
ker$_C$
IRES
mCherry
3LTR
형질도입
-선택
+선택
FACS
FACS
BFP+/mCherry+
BFP+/mCherry+

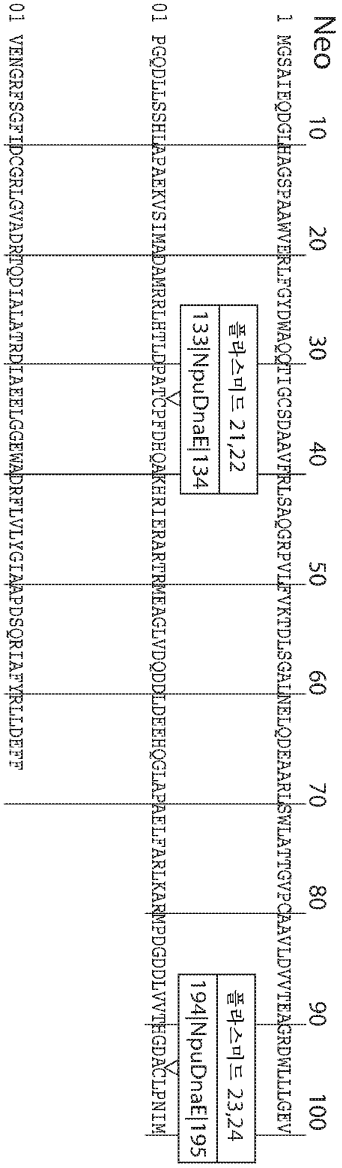
도면2a


Hygro
플라스미드 7,8
52|NpuDnaE|53
플라스미드 64,65
69|NpuDnaE|^C70
플라스미드 3,4
89|NpuDnaE|90
플라스미드 66,67
131|NpuDnaE|^C132
플라스미드 68,69
171|NpuDnaE|^C172
플라스미드 5,6
200|SspDnaB|201
플라스미드 70,71
218|NpuDnaE|^C219
플라스미드 9,10
240|NpuDnaE|241
플라스미드 72,73
259|NpuDnaE|^C260
플라스미드 74,75
277|NpuDnaE|^C278
플라스미드 11,12
292|NpuDnaE|293

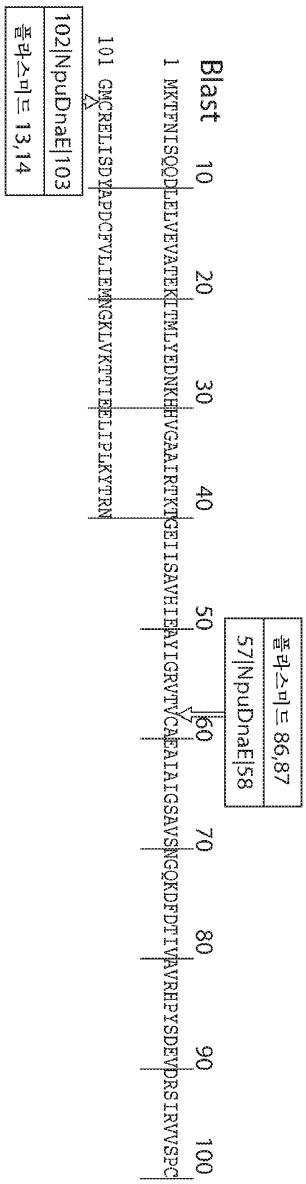
도면2b


Puro
1 MTEYKPTVRLATRDDVPRAVRTLAAAFADYPATRHTVDPDRHIERVTELQELFLTRVGLDIGKVWVADDGAAVAVWTTPESVEAGAVFAEIGPRMAELSG
101 SRLAAQQMEGLLAPHRPKEPAWFLATVGVSPDHQGKGLGSAVVLPGVEAAERAGVPAFLETSAPRNLPFYERLGFTVTADVEVPEGPRTWCMTRKPGA
플라스미드 76,77
32|NpuDnaE|^C33
플라스미드 78,79
84|NpuDnaE|^C85
플라스미드 19,20
100|SspDnaB|101
플라스미드 17,18
119|NpuDnaE|^C120
플라스미드 80,81
137|NpuDnaE|^C138
플라스미드 82,83
158|NpuDnaE|^C159
플라스미드 84,85
180|NpuDnaE|^C181

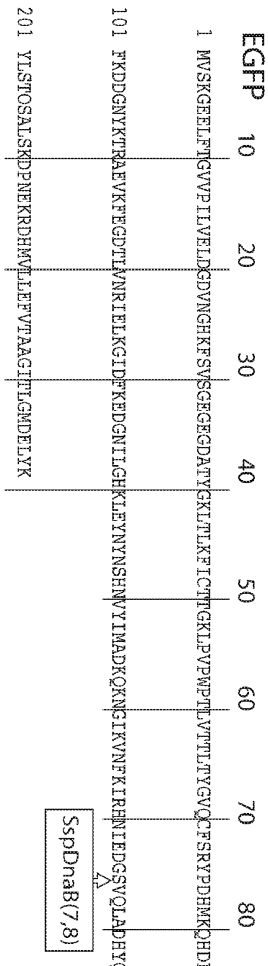
도면2c


Neo
10
20
30
40
50
60
70
80
90
100
1 MGSAIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGRPVLFVKTDLSGALNELQDEAARLSWLATTGVPCAAVLDVVTEAGRDWLLLGEV
101 PGQDLLSSHLAPAEKVSIMADAMRRLHTLDPATCPFDEQAKHRIERARTRMEAGLVDQDDLDEEHQGLAPAELFARLKARMPDGDDLVVTHGDACLPNIM
201 VENGRFSGFIDCGRLGVADRYQDIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLDEFF
플라스미드 21,22
133|NpuDnaE|134
플라스미드 23,24
194|NpuDnaE|195

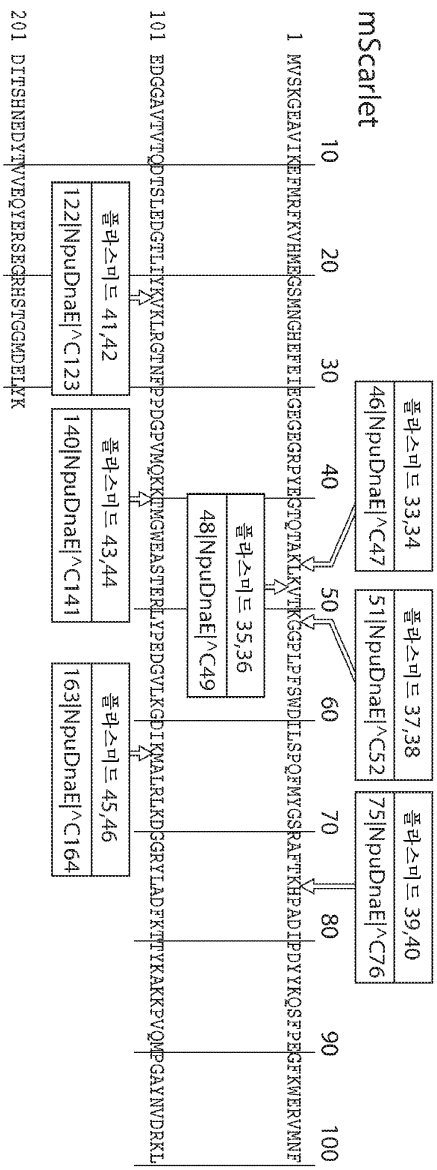
도면2d


Blast
1 MKTFNISQQDLELVEVATEKLTMLYEDNKHHVGAAIRTKTGEIISAVHIEAYIGRVTVCAEAIAIGSAVSNGQKDFDTIVAVRHPYSDEVDRSIRVVSPC
101 GMCRELISDYAPDCFVLIEMNGKLVKTTIEELIPLKYTRN
102|NpuDnaE|103
플라스미드 13,14
57|NpuDnaE|58
플라스미드 86,87

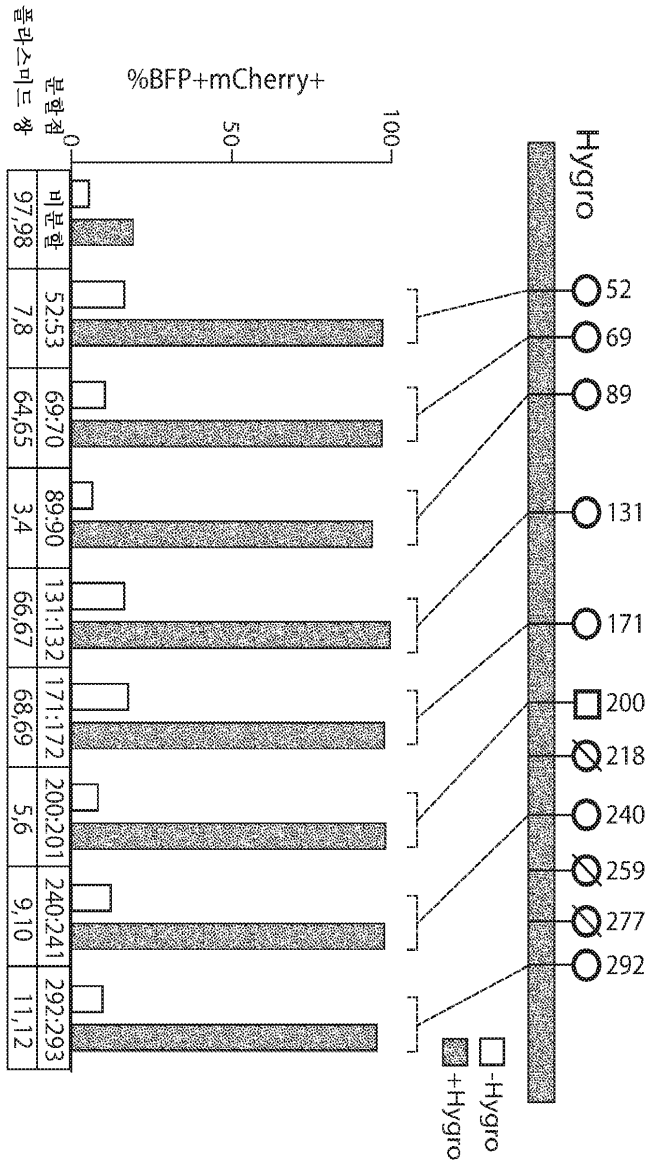
도면2e


EGFP
10
20
30
40
50
60
70
80
90
100
1 MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIF
101 FKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH
201 YLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK
SspDnaB(7,8)

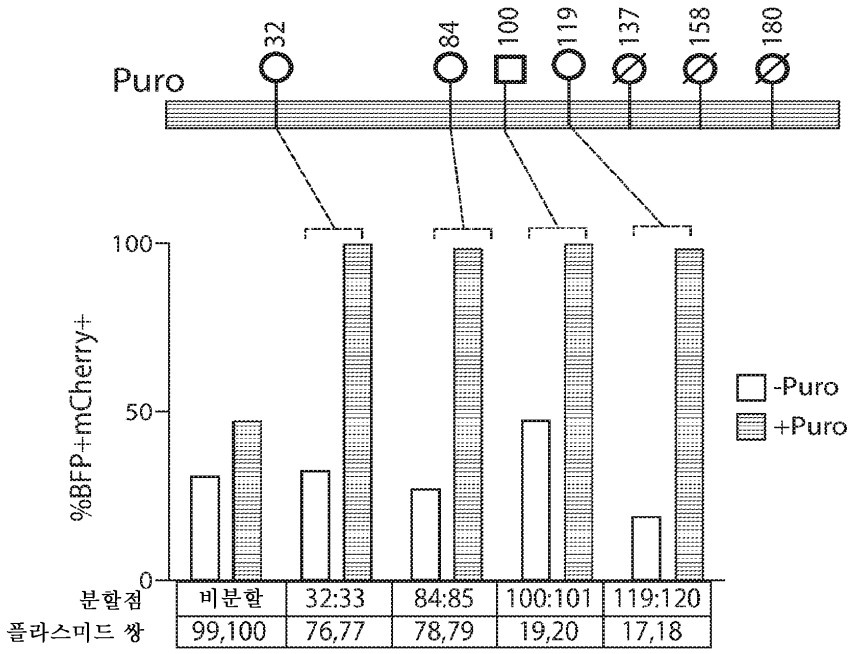
도면2f


mScarlet
1 MVSKGEAVIKEFMRFKVHMEGSMNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFSWDILSPQFMYGSRAFTKHPADIPDYYKQSFPEGFKWERVMNF
101 EDGGAVTVTQDTSLEDGTLIYKVKLRGTNFPPDGPVMQKKTMGWEASTERLYPEDGVLKGDIKMALRLKDGGRYLADFKTTYKAKKPVQMPGAYNVDRKL
201 DITSHNEDYTVVEQYERSEGRHSTGGMDELYK
46|NpuDnaE|^C47
플라스미드 33,34
51|NpuDnaE|^C52
플라스미드 37,38
75|NpuDnaE|^C76
플라스미드 39,40
48|NpuDnaE|^C49
플라스미드 35,36
122|NpuDnaE|^C123
플라스미드 41,42
140|NpuDnaE|^C141
플라스미드 43,44
163|NpuDnaE|^C164
플라스미드 45,46

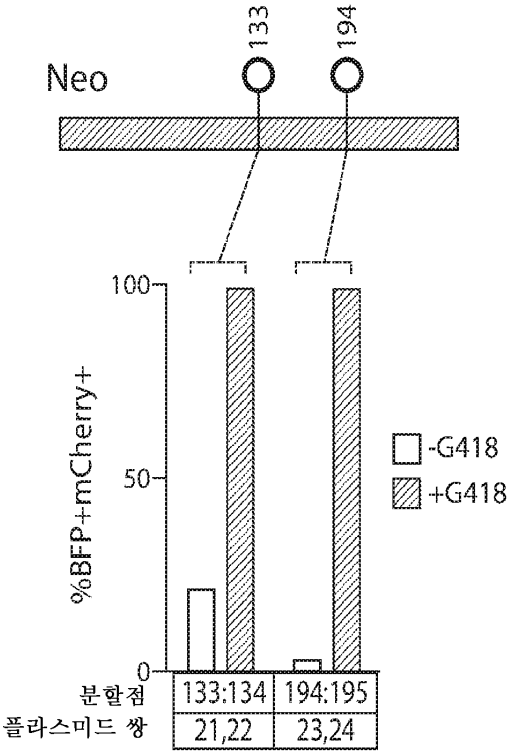
도면3


%BFP+mCherry+
0
50
100
Hygro
52
69
89
131
171
200
218
240
259
277
292
-Hygro
+Hygro
플라스미드 쌍
분할점
비분할
52:53
69:70
89:90
131:132
171:172
200:201
240:241
292:293
97,98
7,8
64,65
3,4
66,67
68,69
5,6
9,10
11,12

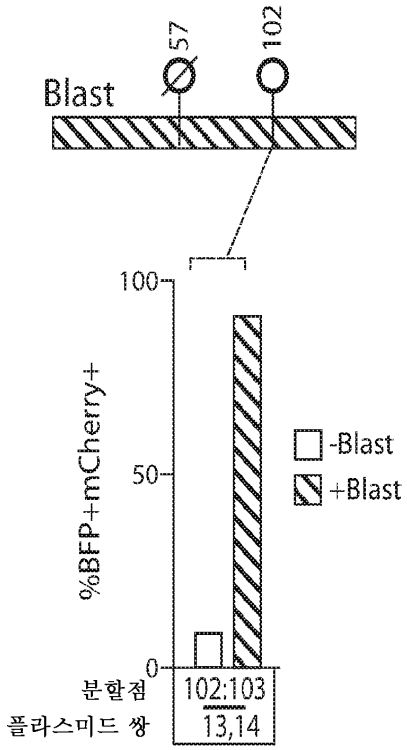
도면4


Puro
32
84
100
119
137
158
180
100
50
0
%BFP+mCherry+
-Puro
+Puro
분할점
비분할
32:33
84:85
100:101
119:120
플라스미드 쌍
99,100
76,77
78,79
19,20
17,18

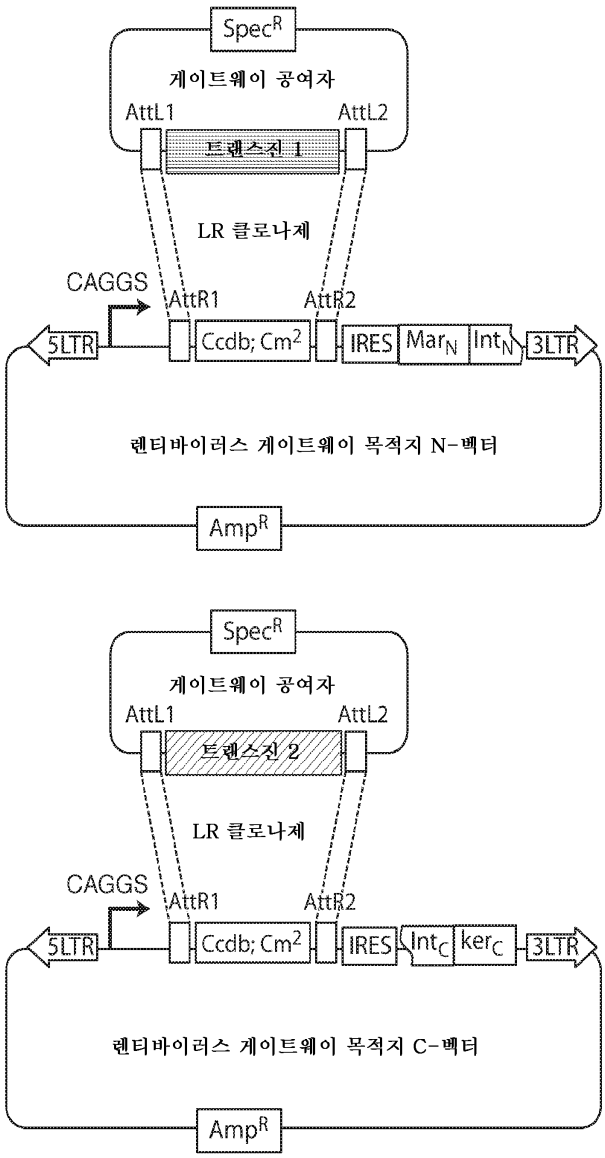
도면5


Neo
133
194
100
50
0
%BFP+mCherry+
-G418
+G418
분할점
133:134
194:195
플라스미드 쌍
21,22
23,24

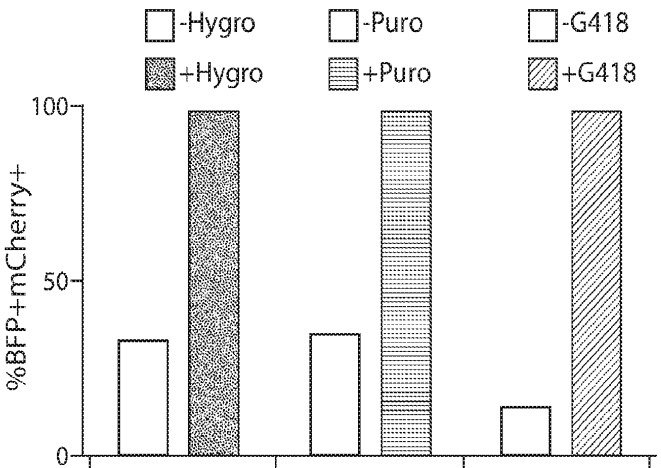
도면6


Blast
57
102
100
50
0
%BFP+mCherry+
-Blast
+Blast
분할점
102;103
플라스미드 쌍
13,14

도면7a

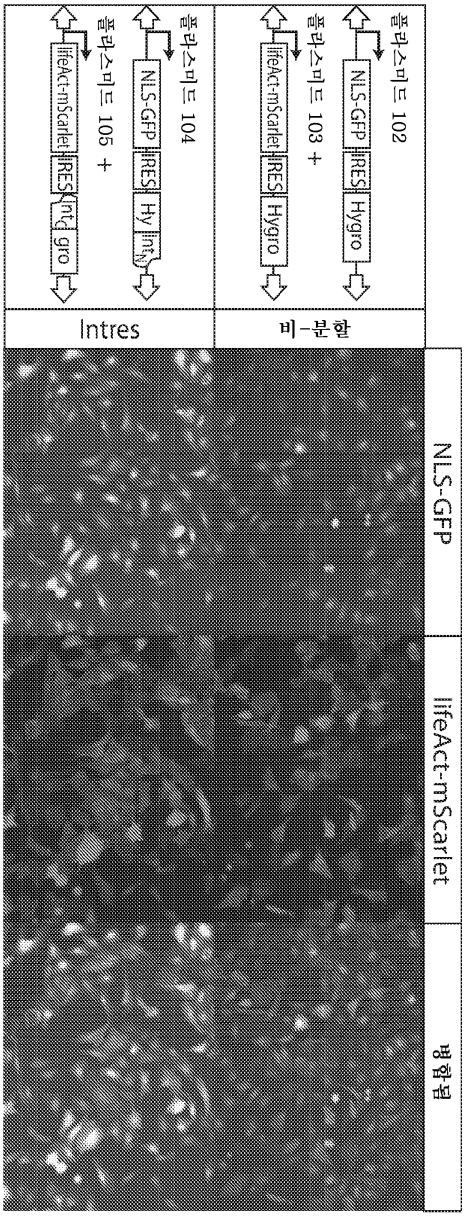

Spec^R
게이트웨이 공여자
AttL1
AttL2
트랜스진 1
LR 클로나제
CAGGS
AttR1
AttR2
5LTR
Ccdb; Cm^2
IRES
Mar_N
Int_N
3LTR
렌티바이러스 게이트웨이 목적지 N-벡터
Amp^R
Spec^R
게이트웨이 공여자
AttL1
AttL2
트랜스진 2
LR 클로나제
CAGGS
AttR1
AttR2
5LTR
Ccdb; Cm^2
IRES
Int_C
ker_C
3LTR
렌티바이러스 게이트웨이 목적지 C-벡터
Amp^R

도면7b

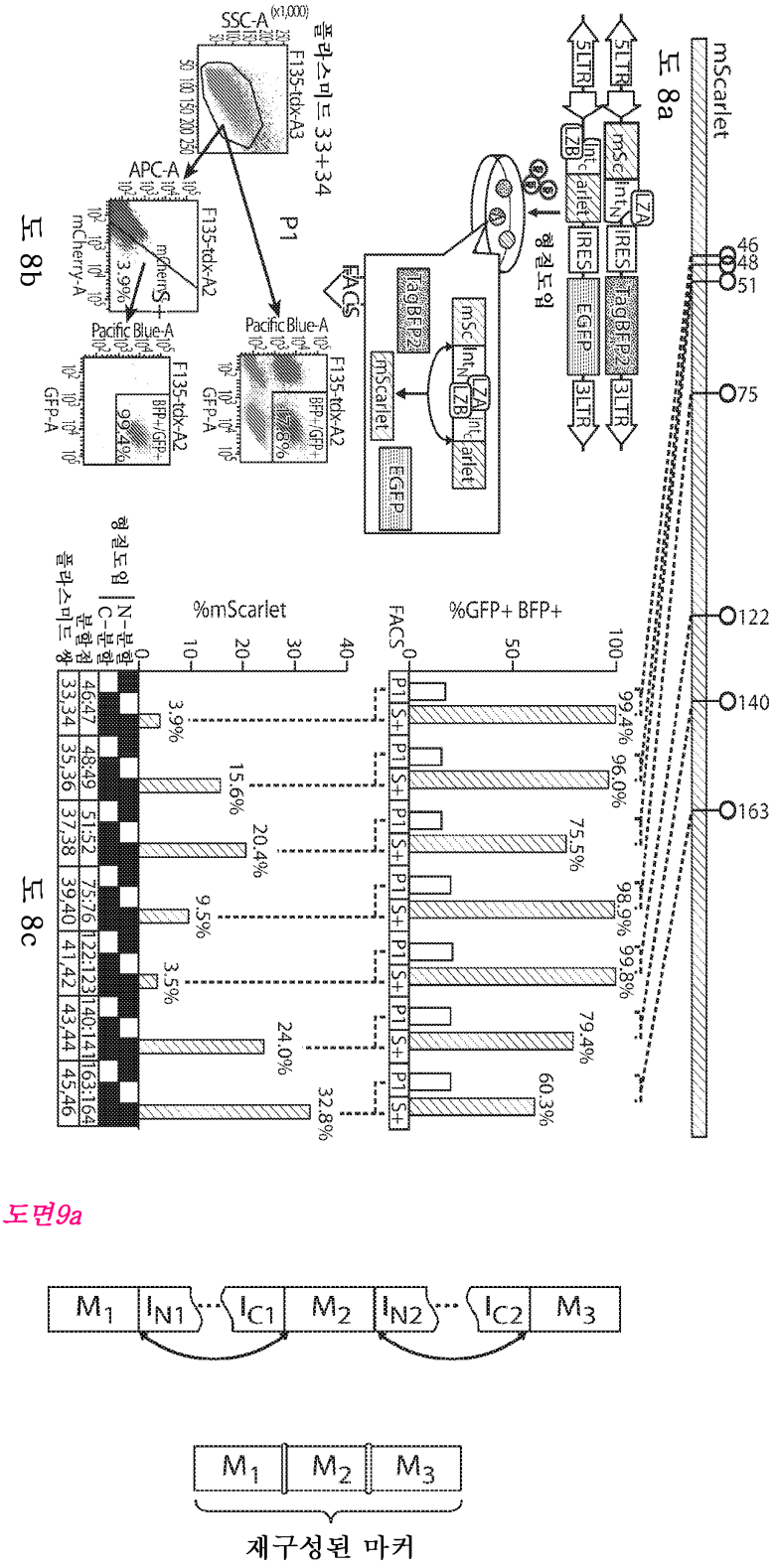


내성	Hygro	Puro	Neo
목적지 플라스미드	49,50	53,54	57,58
최종 플라스미드	51,52	55,56	59,60

도면7c


플라스미드 102
NLS-GFP
IRES
Hygro
플라스미드 103 +
lifeAct-mScarlet
IRES
Hygro
비-분할
플라스미드 104
NLS-GFP
IRES
Hy
플라스미드 105 +
lifeAct-mScarlet
IRES
gro
Intres
NLS-GFP
lifeAct-mScarlet
병합됨

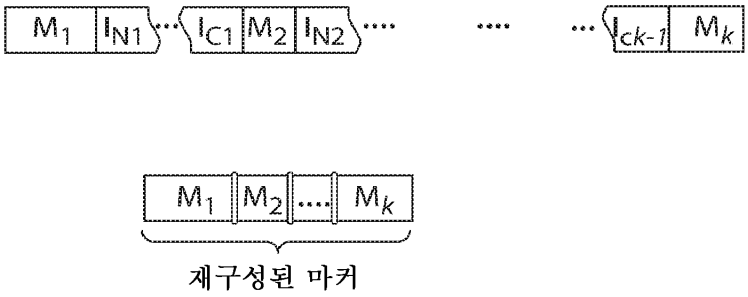
도면8


도 8a
5LTR
LZA
IRES
EGFP
3LTR
mScarlet
46
48
51
75
122
140
163
형질도입
FACS
P1
플라스미드 33+34
SSC-A
F135-tdx-A3
APC-A
mCherry-A
F135-tdx-A2
3.9%
Pacific Blue-A
GFP-A
BFP-/GFP-
99.4%
BFP+/GFP+
97.8%
도 8b
%mScarlet
%GFP+ BFP+
N-분할
C-분할
분할 쌍
33,34
46:47
35,36
48:49
37,38
51:52
39,40
75:76
41,42
122:123
43,44
140:141
45,46
163:164
3.9%
15.6%
20.4%
9.5%
3.5%
24.0%
32.8%
99.4%
96.0%
75.5%
98.9%
99.8%
79.4%
60.3%
P1S+
도 8c

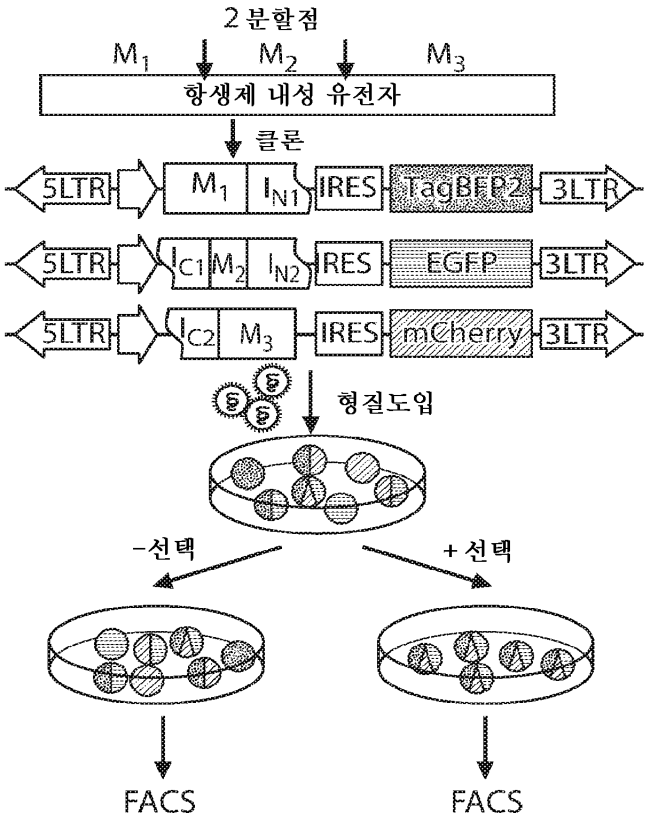
도면9a

M1
IN1
IC1
M2
IN2
IC2
M3
M1
M2
M3
재구성된 마커

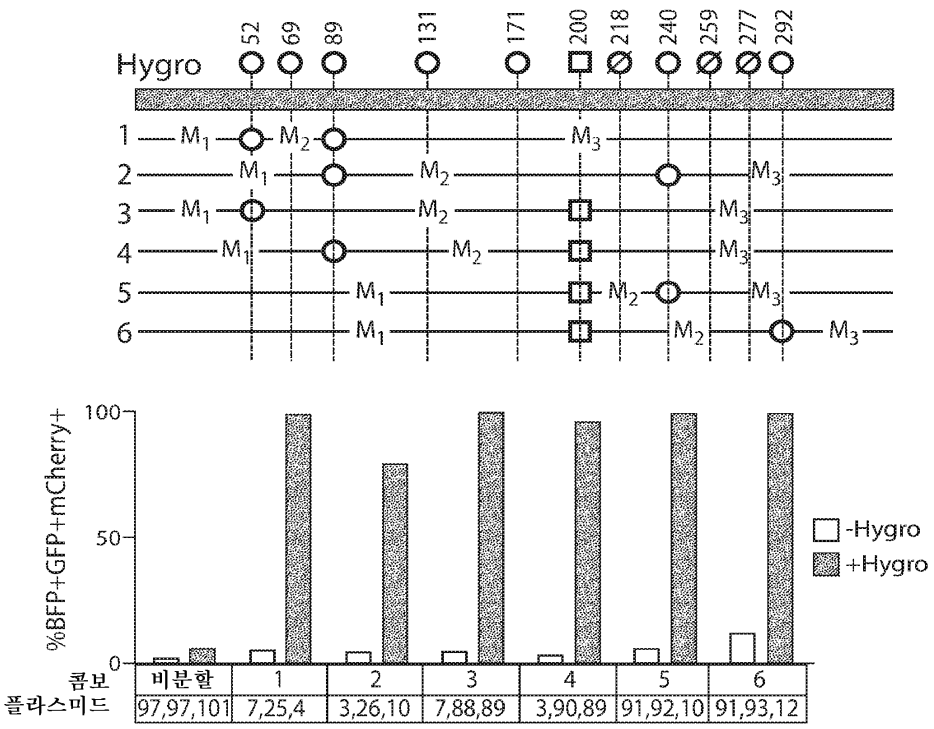
도면9b


M1
IN1
IC1
M2
IN2
Ick-1
Mk
재구성된 마커

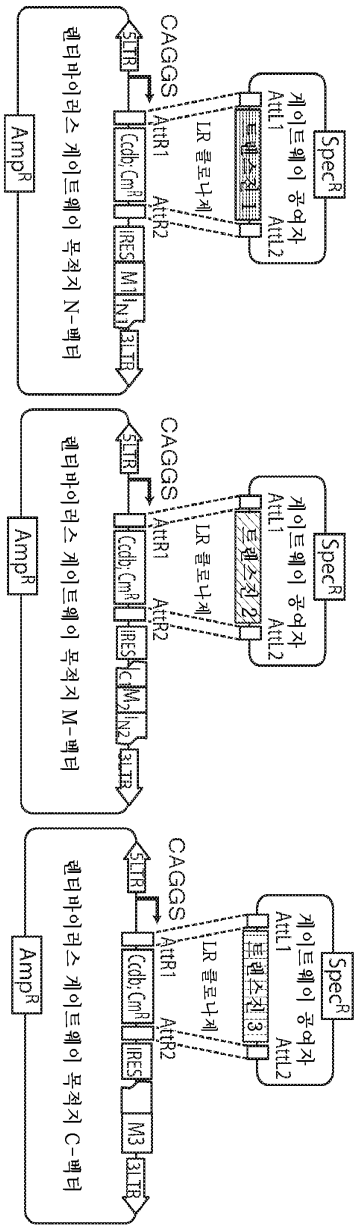
도면9c


2 분할점
M1
M2
M3
항생제 내성 유전자
클론
5LTR
IRES
TagBFP2
3LTR
EGFP
mCherry
형질도입
-선택
+선택
FACS
FACS

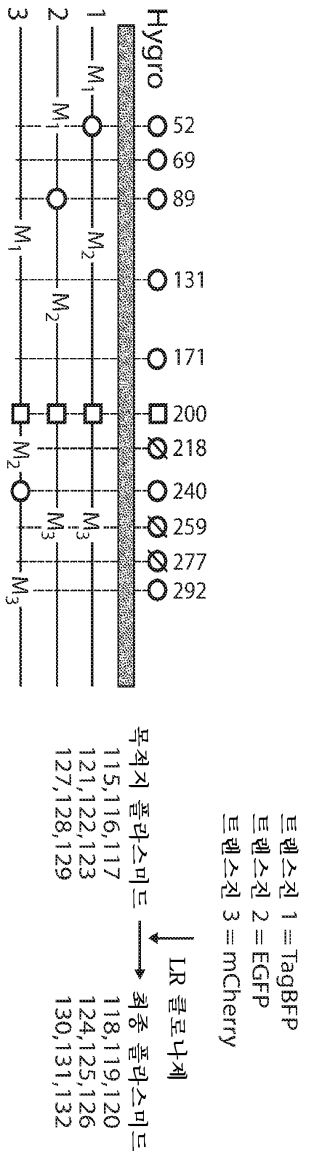
도면9d


Hygro
52
69
89
131
171
200
218
240
259
277
292
1
2
3
4
5
6
M1
M2
M3
%BFP+GFP+mCherry+
100
50
0
-Hygro
+Hygro
콤보
플라스미드
비분할
1
2
3
4
5
6
97,97,101
7,25,4
3,26,10
7,88,89
3,90,89
91,92,10
91,93,12

도면10a


렌티바이러스 게이트웨이 목적지 N-벡터
5LTR
CAGGS
AttR1
Ccdb, CmR
AttR2
IRES
M1
N1
3LTR
AmpR
LR 클로나제
게이트웨이 공여자
AttL1
AttL2
SpecR
트랜스진
렌티바이러스 게이트웨이 목적지 M-벡터
5LTR
CAGGS
AttR1
Ccdb, CmR
AttR2
IRES
M2
N2
3LTR
AmpR
LR 클로나제
게이트웨이 공여자
AttL1
AttL2
SpecR
트랜스진 2
렌티바이러스 게이트웨이 목적지 C-벡터
5LTR
CAGGS
AttR1
Ccdb, CmR
AttR2
IRES
M3
3LTR
AmpR
LR 클로나제
게이트웨이 공여자
AttL1
AttL2
SpecR
트랜스진 3

도면10b

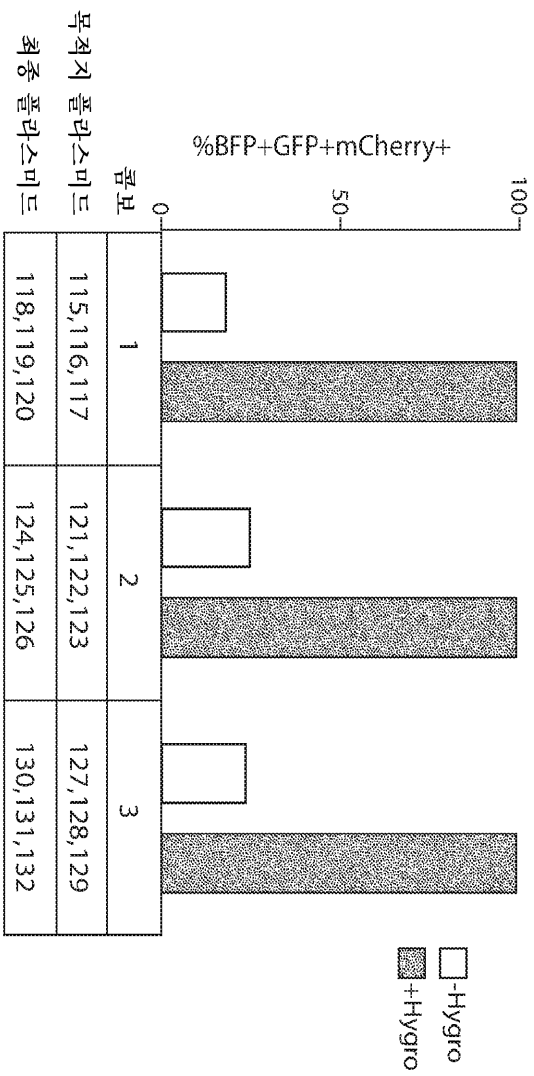


트랜스진 1 = TagBFP
트랜스진 2 = EGFP
트랜스진 3 = mCherry

목적지 플라스미드 ⟷ 최종 플라스미드
LR 클로나제

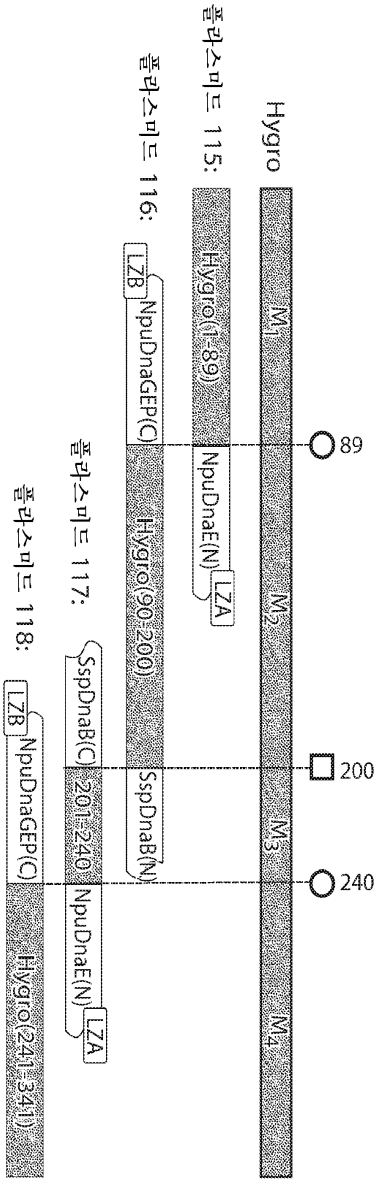
목적지 플라스미드	최종 플라스미드
115,116,117	118,119,120
121,122,123	124,125,126
127,128,129	130,131,132

도면10c

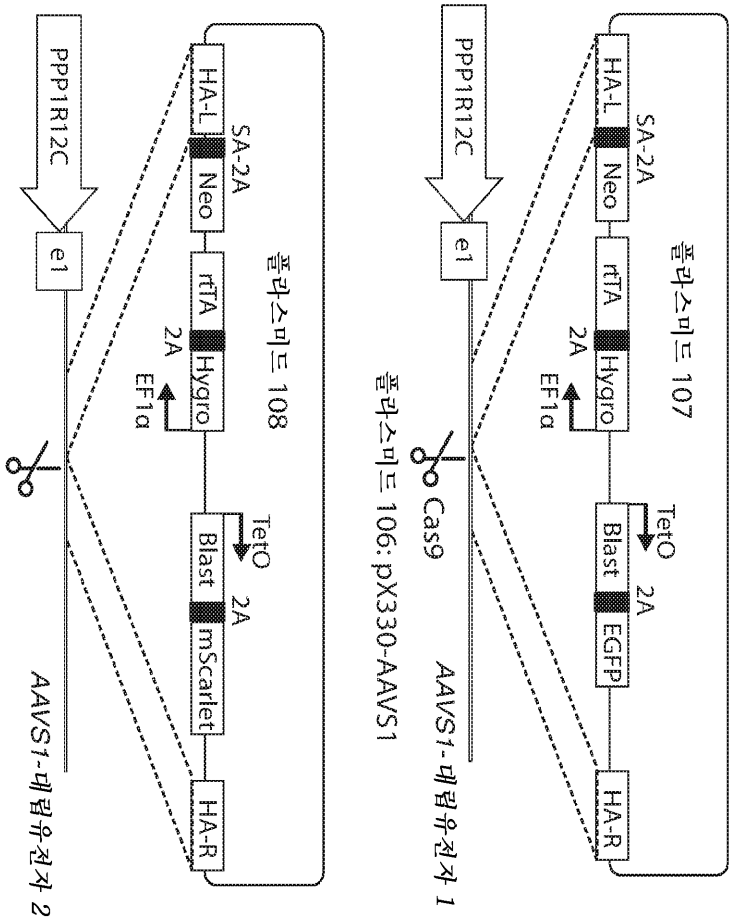


콤보	목적지 플라스미드	최종 플라스미드
1	115,116,117	118,119,120
2	121,122,123	124,125,126
3	127,128,129	130,131,132

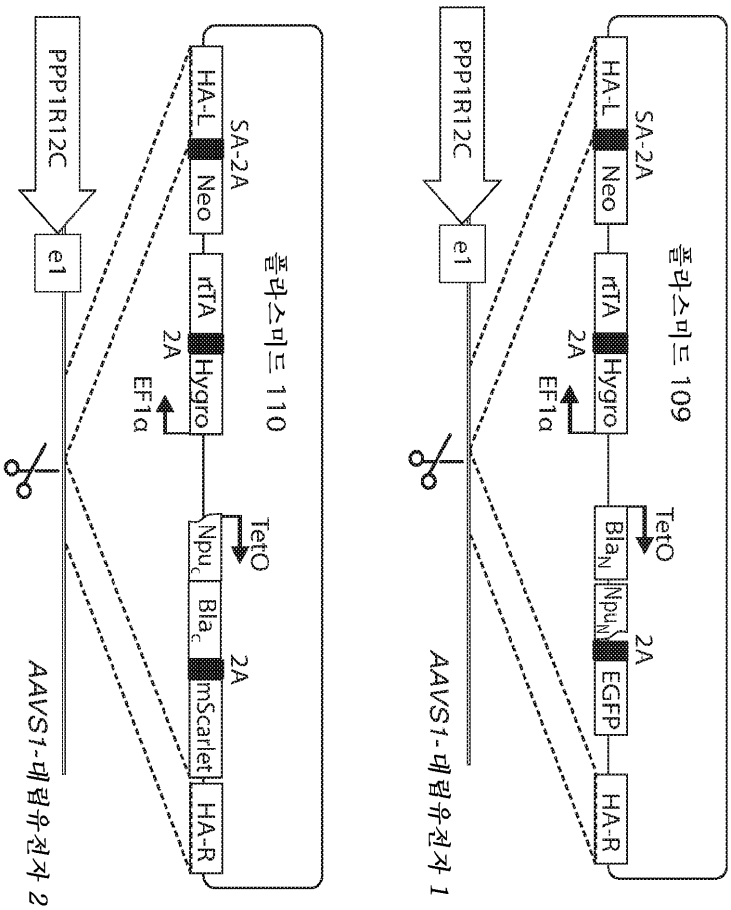
도면11


Hygro
M1
M2
M3
M4
89
200
240
플라스미드 115:
Hygro(1-89)
NpuDnaE(N)
LZA
플라스미드 116:
LZB
NpuDnaGEP(C)
Hygro(90-200)
SspDnaB(N)
플라스미드 117:
SspDnaB(C)
201-240
NpuDnaE(N)
LZA
플라스미드 118:
LZB
NpuDnaGEP(C)
Hygro(241-341)

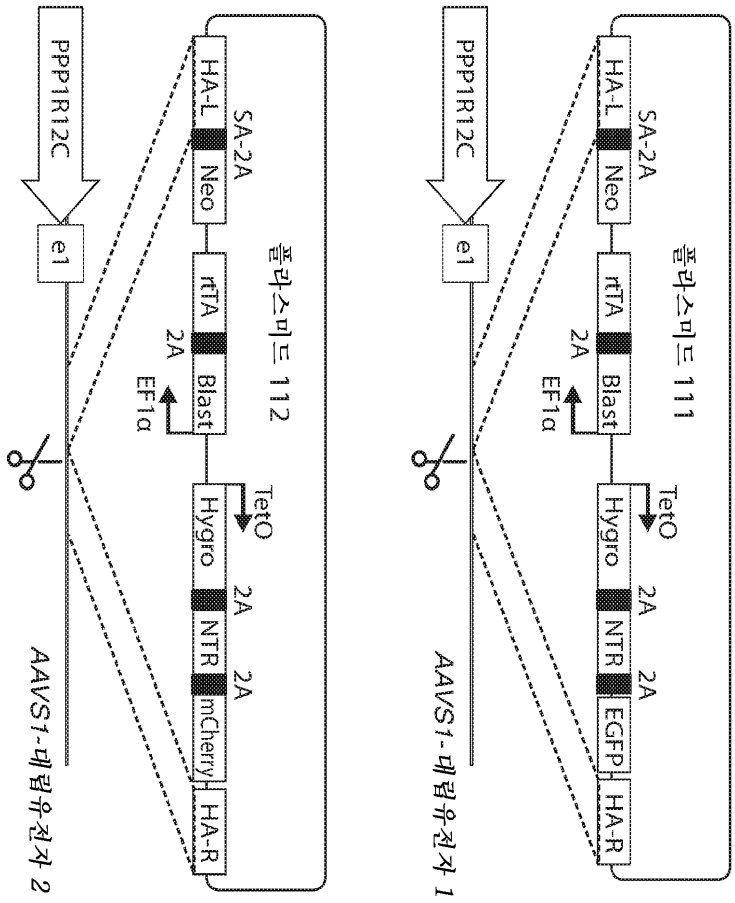
도면12a


PPP1R12C
e1
Cas9
AAVS1-대립유전자 1
플라스미드 106: pX330-AAVS1
플라스미드 107
HA-L
SA-2A
Neo
rtTA
2A
Hygro
EF1α
TetO
2A
Blast
EGFP
HA-R
PPP1R12C
e1
AAVS1-대립유전자 2
플라스미드 108
HA-L
SA-2A
Neo
rtTA
2A
Hygro
EF1α
TetO
2A
Blast
mScarlet
HA-R

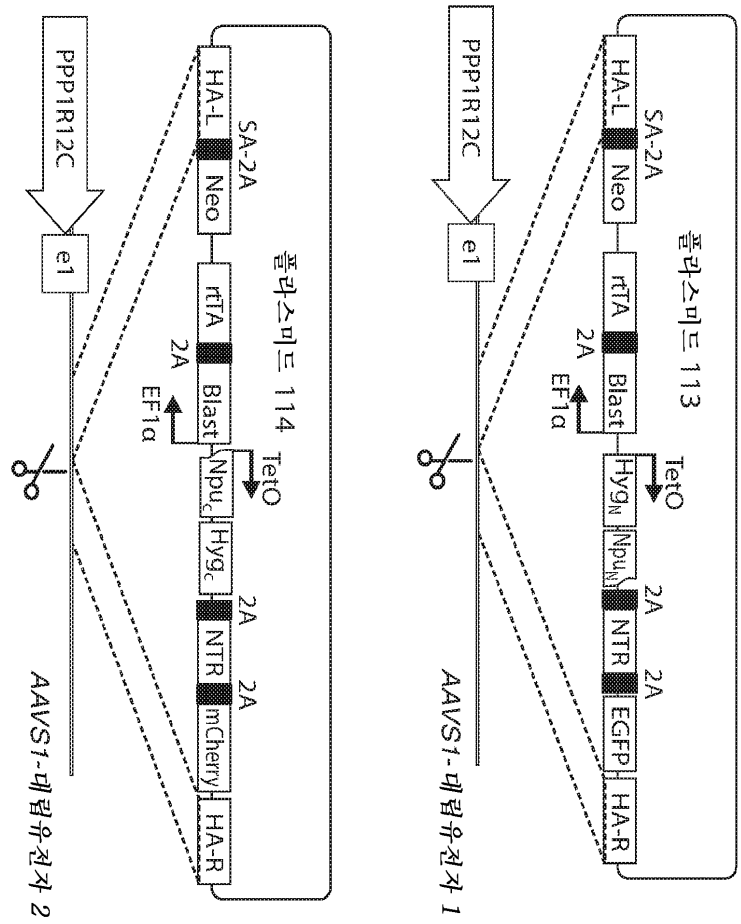
도면12b


PPP1R12C
e1
AAVS1-대립유전자 2
HA-L
SA-2A
Neo
rtTA
2A
Hygro
EF1α
플라스미드 110
TetO
Npu$_C$
Bla$_C$
2A
mScarlet
HA-R
PPP1R12C
e1
AAVS1-대립유전자 1
HA-L
SA-2A
Neo
rtTA
2A
Hygro
EF1α
플라스미드 109
TetO
Bla$_N$
Npu$_N$
2A
EGFP
HA-R

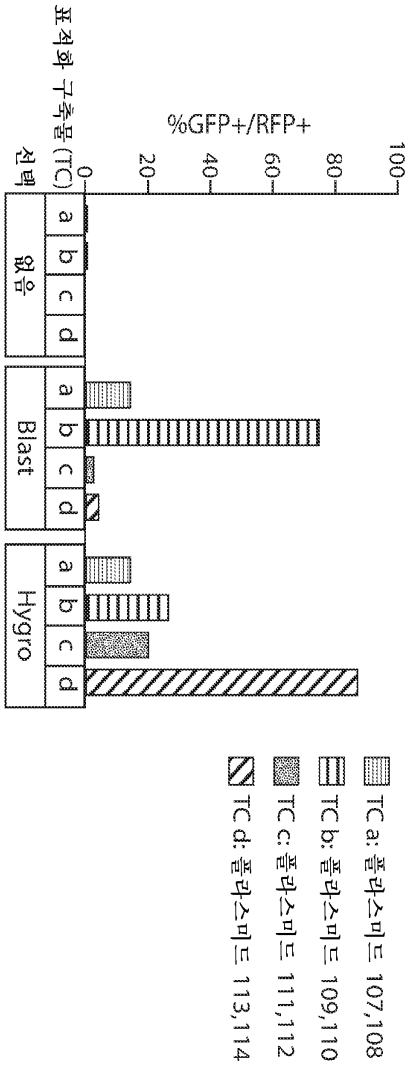
도면12c


PPP1R12C
e1
AAVS1-대립유전자 1
플라스미드 111
SA-2A
HA-L
Neo
rtTA
2A
Blast
EF1α
TetO
Hygro
2A
NTR
2A
EGFP
HA-R
PPP1R12C
e1
AAVS1-대립유전자 2
플라스미드 112
SA-2A
HA-L
Neo
rtTA
2A
Blast
EF1α
TetO
Hygro
2A
NTR
2A
mCherry
HA-R

도면12d


PPP1R12C
e1
AAVS1-대립유전자 2
플라스미드 114
SA-2A
HA-L
Neo
rtTA
2A
EF1α
Blast
TetO
Npu$_C$
Hyg$_C$
2A
NTR
2A
mCherry
HA-R
PPP1R12C
e1
AAVS1-대립유전자 1
플라스미드 113
SA-2A
HA-L
Neo
rtTA
2A
EF1α
Blast
TetO
Hyg$_N$
Npu$_N$
2A
NTR
2A
EGFP
HA-R

도면12e



서 열 목 록

SEQUENCE LISTING

<110> The Jackson Laboratory

<120> TRANSGENIC SELECTION METHODS AND COMPOSITIONS

<130> J0227.70007WO00

<140> Not Yet Assigned

<141> Concurrently Herewith

<150> US 62/624,629

<151> 2018-01-31

<150> US 62/616,281

<151> 2018-01-11

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<151> 2017-12-20

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<170> PatentIn version 3.5

<210> 1

<211> 341

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

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400> 1

Met Lys Lys Pro Glu Leu Thr Ala Thr Ser Val Glu Lys Phe Leu Ile
1 5 10 15

Glu Lys Phe Asp Ser Val Ser Asp Leu Met Gln Leu Ser Glu Gly Glu
20 25 30

Glu Ser Arg Ala Phe Ser Phe Asp Val Gly Gly Arg Gly Tyr Val Leu
35 40 45

Arg Val Asn Ser Cys Ala Asp Gly Phe Tyr Lys Asp Arg Tyr Val Tyr
50 55 60

Arg His Phe Ala Ser Ala Ala Leu Pro Ile Pro Glu Val Leu Asp Ile
65 70 75 80

Gly Glu Phe Ser Glu Ser Leu Thr Tyr Cys Ile Ser Arg Arg Ala Gln
85 90 95

Gly Val Thr Leu Gln Asp Leu Pro Glu Thr Glu Leu Pro Ala Val Leu
100 105 110

Gln Pro Val Ala Glu Ala Met Asp Ala Ile Ala Ala Ala Asp Leu Ser
115 120 125

Gln Thr Ser Gly Phe Gly Pro Phe Gly Pro Gln Gly Ile Gly Gln Tyr
130 135 140

Thr Thr Trp Arg Asp Phe Ile Cys Ala Ile Ala Asp Pro His Val Tyr
145 150 155 160

His Trp Gln Thr Val Met Asp Asp Thr Val Ser Ala Ser Val Ala Gln

165 170 175

Ala Leu Asp Glu Leu Met Leu Trp Ala Glu Asp Cys Pro Glu Val Arg

180 185 190

His Leu Val His Ala Asp Phe Gly Ser Asn Asn Val Leu Thr Asp Asn

195 200 205

Gly Arg Ile Thr Ala Val Ile Asp Trp Ser Glu Ala Met Phe Gly Asp

210 215 220

Ser Gln Tyr Glu Val Ala Asn Ile Phe Phe Trp Arg Pro Trp Leu Ala

225 230 235 240

Cys Met Glu Gln Gln Thr Arg Tyr Phe Glu Arg Arg His Pro Glu Leu

245 250 255

Ala Gly Ser Pro Arg Leu Arg Ala Tyr Met Leu Arg Ile Gly Leu Asp

260 265 270

Gln Leu Tyr Gln Ser Leu Val Asp Gly Asn Phe Asp Asp Ala Ala Trp

275 280 285

Ala Gln Gly Arg Cys Asp Ala Ile Val Arg Ser Gly Ala Gly Thr Val

290 295 300

Gly Arg Thr Gln Ile Ala Arg Arg Ser Ala Ala Val Trp Thr Asp Gly

305 310 315 320

Cys Val Glu Val Leu Ala Asp Ser Gly Asn Arg Arg Pro Ser Thr Arg

325 330 335

Pro Arg Ala Lys Glu

340

<210> 2

<211> 199

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

<400> 2

Met Thr Glu Tyr Lys Pro Thr Val Arg Leu Ala Thr Arg Asp Asp Val

1 5 10 15

Pro Arg Ala Val Arg Thr Leu Ala Ala Ala Phe Ala Asp Tyr Pro Ala

20 25 30

Thr Arg His Thr Val Asp Pro Asp Arg His Ile Glu Arg Val Thr Glu
35 40 45
Leu Gln Glu Leu Phe Leu Thr Arg Val Gly Leu Asp Ile Gly Lys Val
50 55 60
Trp Val Ala Asp Asp Gly Ala Ala Val Ala Val Trp Thr Thr Pro Glu
65 70 75 80
Ser Val Glu Ala Gly Ala Val Phe Ala Glu Ile Gly Pro Arg Met Ala
85 90 95

Glu Leu Ser Gly Ser Arg Leu Ala Ala Gln Gln Gln Met Glu Gly Leu
100 105 110
Leu Ala Pro His Arg Pro Lys Glu Pro Ala Trp Phe Leu Ala Thr Val
115 120 125
Gly Val Ser Pro Asp His Gln Gly Lys Gly Leu Gly Ser Ala Val Val
130 135 140
Leu Pro Gly Val Glu Ala Ala Glu Arg Ala Gly Val Pro Ala Phe Leu
145 150 155 160

Glu Thr Ser Ala Pro Arg Asn Leu Pro Phe Tyr Glu Arg Leu Gly Phe
165 170 175
Thr Val Thr Ala Asp Val Glu Val Pro Glu Gly Pro Arg Thr Trp Cys
180 185 190
Met Thr Arg Lys Pro Gly Ala
195

<210> 3
<211> 264
<212> PRT
<213> Artificial Sequence
<220><223> Synthetic Polypeptide
<400> 3
Met Ile Glu Gln Asp Gly Leu His Ala Gly Ser Pro Ala Ala Trp Val
1 5 10 15

Glu Arg Leu Phe Gly Tyr Asp Trp Ala Gln Gln Thr Ile Gly Cys Ser
20 25 30
Asp Ala Ala Val Phe Arg Leu Ser Ala Gln Gly Arg Pro Val Leu Phe
35 40 45
Val Lys Thr Asp Leu Ser Gly Ala Leu Asn Glu Leu Gln Asp Glu Ala
50 55 60
Ala Arg Leu Ser Trp Leu Ala Thr Thr Gly Val Pro Cys Ala Ala Val
65 70 75 80

Leu Asp Val Val Thr Glu Ala Gly Arg Asp Trp Leu Leu Leu Gly Glu
85 90 95
Val Pro Gly Gln Asp Leu Leu Ser Ser His Leu Ala Pro Ala Glu Lys
100 105 110
Val Ser Ile Met Ala Asp Ala Met Arg Arg Leu His Thr Leu Asp Pro
115 120 125
Ala Thr Cys Pro Phe Asp His Gln Ala Lys His Arg Ile Glu Arg Ala
130 135 140

Arg Thr Arg Met Glu Ala Gly Leu Val Asp Gln Asp Asp Leu Asp Glu
145 150 155 160
Glu His Gln Gly Leu Ala Pro Ala Glu Leu Phe Ala Arg Leu Lys Ala
165 170 175
Arg Met Pro Asp Gly Glu Asp Leu Val Val Thr His Gly Asp Ala Cys
180 185 190
Leu Pro Asn Ile Met Val Glu Asn Gly Arg Phe Ser Gly Phe Ile Asp
195 200 205

Cys Gly Arg Leu Gly Val Ala Asp Arg Tyr Gln Asp Ile Ala Leu Ala
210 215 220
Thr Arg Asp Ile Ala Glu Glu Leu Gly Gly Glu Trp Ala Asp Arg Phe
225 230 235 240
Leu Val Leu Tyr Gly Ile Ala Ala Pro Asp Ser Gln Arg Ile Ala Phe
245 250 255
Tyr Arg Leu Leu Asp Glu Phe Phe
260

<210> 4

<211> 140

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

<400> 4

Met Lys Thr Phe Asn Ile Ser Gln Gln Asp Leu Glu Leu Val Glu Val
1 5 10 15

Ala Thr Glu Lys Ile Thr Met Leu Tyr Glu Asp Asn Lys His His Val
20 25 30

Gly Ala Ala Ile Arg Thr Lys Thr Gly Glu Ile Ile Ser Ala Val His
35 40 45

Ile Glu Ala Tyr Ile Gly Arg Val Thr Val Cys Ala Glu Ala Ile Ala
50 55 60

Ile Gly Ser Ala Val Ser Asn Gly Gln Lys Asp Phe Asp Thr Ile Val
65 70 75 80

Ala Val Arg His Pro Tyr Ser Asp Glu Val Asp Arg Ser Ile Arg Val
85 90 95

Val Ser Pro Cys Gly Met Cys Arg Glu Leu Ile Ser Asp Tyr Ala Pro
100 105 110

Asp Cys Phe Val Leu Ile Glu Met Asn Gly Lys Leu Val Lys Thr Thr
115 120 125

Ile Glu Glu Leu Ile Pro Leu Lys Tyr Thr Arg Asn
130 135 140

<210> 5

<211> 239

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

<400> 5

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly

20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile

35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr

50 55 60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys

65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu

85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu

100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly

115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr

130 135 140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn

145 150 155 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser

165 170 175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly

180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu

195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe

210 215 220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

225 230 235

<210> 6

<211> 232

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

<400> 6

Met Val Ser Lys Gly Glu Ala Val Ile Lys Glu Phe Met Arg Phe Lys
1 5 10 15

Val His Met Glu Gly Ser Met Asn Gly His Glu Phe Glu Ile Glu Gly
20 25 30

Glu Gly Glu Gly Arg Pro Tyr Glu Gly Thr Gln Thr Ala Lys Leu Lys
35 40 45

Val Thr Lys Gly Gly Pro Leu Pro Phe Ser Trp Asp Ile Leu Ser Pro
50 55 60

Gln Phe Met Tyr Gly Ser Arg Ala Phe Thr Lys His Pro Ala Asp Ile
65 70 75 80

Pro Asp Tyr Tyr Lys Gln Ser Phe Pro Glu Gly Phe Lys Trp Glu Arg
85 90 95

Val Met Asn Phe Glu Asp Gly Gly Ala Val Thr Val Thr Gln Asp Thr
100 105 110

Ser Leu Glu Asp Gly Thr Leu Ile Tyr Lys Val Lys Leu Arg Gly Thr
115 120 125

Asn Phe Pro Pro Asp Gly Pro Val Met Gln Lys Lys Thr Met Gly Trp
130 135 140

Glu Ala Ser Thr Glu Arg Leu Tyr Pro Glu Asp Gly Val Leu Lys Gly
145 150 155 160

Asp Ile Lys Met Ala Leu Arg Leu Lys Asp Gly Gly Arg Tyr Leu Ala
165 170 175

Asp Phe Lys Thr Thr Tyr Lys Ala Lys Lys Pro Val Gln Met Pro Gly
180 185 190

Ala Tyr Asn Val Asp Arg Lys Leu Asp Ile Thr Ser His Asn Glu Asp
195 200 205

Tyr Thr Val Val Glu Gln Tyr Glu Arg Ser Glu Gly Arg His Ser Thr
210 215 220

Gly Gly Met Asp Glu Leu Tyr Lys

225 230

<210> 7

<211> 102

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

<400> 7

Cys Leu Ser Tyr Glu Thr Glu Ile Leu Thr Val Glu Tyr Gly Leu Leu
1 5 10 15

Pro Ile Gly Lys Ile Val Glu Lys Arg Ile Glu Cys Thr Val Tyr Ser
20 25 30

Val Asp Asn Asn Gly Asn Ile Tyr Thr Gln Pro Val Ala Gln Trp His
35 40 45

Asp Arg Gly Glu Gln Glu Val Phe Glu Tyr Cys Leu Glu Asp Gly Ser
50 55 60

Leu Ile Arg Ala Thr Lys Asp His Lys Phe Met Thr Val Asp Gly Gln
65 70 75 80

Met Leu Pro Ile Asp Glu Ile Phe Glu Arg Glu Leu Asp Leu Met Arg
85 90 95

Val Asp Asn Leu Pro Asn
100

<210> 8

<211> 35

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

<400> 8

Ile Lys Ile Ala Thr Arg Lys Tyr Leu Gly Lys Gln Asn Val Tyr Asp
1 5 10 15

Ile Gly Val Glu Arg Asp His Asn Phe Ala Leu Lys Asn Gly Phe Ile
20 25 30

Ala Ser Asn

35

<210> 9

<211> 106

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

<400> 9

Cys Ile Ser Gly Asp Ser Leu Ile Ser Leu Ala Ser Thr Gly Lys Arg
1 5 10 15

Val Ser Ile Lys Asp Leu Leu Asp Glu Lys Asp Phe Glu Ile Trp Ala
20 25 30

Ile Asn Glu Gln Thr Met Lys Leu Glu Ser Ala Lys Val Ser Arg Val
35 40 45

Phe Cys Thr Gly Lys Lys Leu Val Tyr Ile Leu Lys Thr Arg Leu Gly
50 55 60

Arg Thr Ile Lys Ala Thr Ala Asn His Arg Phe Leu Thr Ile Asp Gly
65 70 75 80

Trp Lys Arg Leu Asp Glu Leu Ser Leu Lys Glu His Ile Ala Leu Pro
85 90 95

Arg Lys Leu Glu Ser Ser Ser Leu Gln Leu
100 105

<210> 10

<211> 48

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

<400> 10

Ser Pro Glu Ile Glu Lys Leu Ser Gln Ser Asp Ile Tyr Trp Asp Ser
1 5 10 15

Ile Val Ser Ile Thr Glu Thr Gly Val Glu Glu Val Phe Asp Leu Thr
20 25 30

Val Pro Gly Pro His Asn Phe Val Ala Asn Asp Ile Ile Val His Asn

35 40 45

<210> 11

<211> 139

<212> PRT

<213> Artificial Sequence

<220><223> Synthetic Polypeptide

<400> 11

Cys Leu Ser Tyr Glu Thr Glu Ile Leu Thr Val Glu Tyr Gly Leu Leu
1 5 10 15

Pro Ile Gly Lys Ile Val Glu Lys Arg Ile Glu Cys Thr Val Tyr Ser
20 25 30

Val Asp Asn Asn Gly Asn Ile Tyr Thr Gln Pro Val Ala Gln Trp His

35 40 45

Asp Arg Gly Glu Gln Glu Val Phe Glu Tyr Cys Leu Glu Asp Gly Ser
50 55 60

Leu Ile Arg Ala Thr Lys Asp His Lys Phe Met Thr Val Asp Gly Gln
65 70 75 80

Met Leu Pro Ile Asp Glu Ile Phe Glu Arg Glu Leu Asp Leu Met Arg
85 90 95

Val Asp Asn Leu Pro Asn Gly Gly Gly Gly Ser Gly Ser Ala Gln Leu

100 105 110

Glu Lys Glu Leu Gln Ala Leu Glu Lys Lys Leu Ala Gln Leu Glu Trp
115 120 125

Glu Asn Gln Ala Leu Glu Lys Glu Leu Ala Gln
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Asp Arg Gly Glu Gln Glu Val Leu Glu Tyr Glu Leu Glu Asp Gly Ser

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Val Ile Arg Ala Thr Ser Asp His Arg Phe Leu Thr Thr Asp Tyr Gln
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Leu Leu Ala Ile Glu Glu Ile Phe Ala Arg Gln Leu Asp Leu Leu Thr
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Lys Val Ser Arg Val Phe Cys Thr Gly Lys Lys Leu Val Tyr Ile Leu

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Lys Thr Arg Leu Gly Arg Thr Ile Lys Ala Thr Ala Asn His Arg Phe
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Leu Thr Ile Asp Gly Trp Lys Arg Leu Asp Glu Leu Ser Leu Lys Glu
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His Ile Ala Leu Pro Arg Lys Leu Glu Ser Ser Ser Leu Gln Leu Ser
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Pro Glu Ile Glu Lys Leu Ser Gln Ser Asp Ile Tyr Trp Asp Ser Ile

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Ile Leu Thr Val Glu Tyr Gly Leu Leu Pro Ile Gly Lys Ile Val Glu

100 105 110

Lys Arg Ile Glu Cys Thr Val Tyr Ser Val Asp Asn Asn Gly Asn Ile

115 120 125

Tyr Thr Gln Pro Val Ala Gln Trp His Asp Arg Gly Glu Gln Glu Val

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Phe Glu Tyr Cys Leu Glu Asp Gly Ser Leu Ile Arg Ala Thr Lys Asp

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Glu His Ile Ala Leu Pro Arg Lys Leu Glu Ser Ser Ser Leu Gln Leu
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Ile Val Ser Ile Thr Glu Thr Gly Val Glu Glu Val Phe Asp Leu Thr
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Phe Glu Tyr Cys Leu Glu Asp Gly Ser Leu Ile Arg Ala Thr Lys Asp

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Asp Leu Pro Glu Thr Glu Leu Pro Ala Val Leu Gln Pro Val Ala Glu
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Lys Gln Ser Phe Pro Glu Gly Phe Thr Trp Glu Arg Val Thr Thr Tyr

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Glu Asp Gly Gly Val Leu Thr Ala Thr Gln Asp Thr Ser Leu Gln Asp

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Gly Cys Leu Ile Tyr Asn Val Lys Ile Arg Gly Val Asn Phe Thr Ser

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Asn Gly Pro Val Met Gln Lys Lys Thr Leu Gly Trp Glu Ala Phe Thr

130 135 140

Glu Thr Leu Tyr Pro Ala Asp Gly Gly Leu Glu Gly Arg Asn Asp Met

145 150 155 160

Ala Leu Lys Leu Val Gly Gly Ser His Leu Ile Ala Asn Ile Lys Thr

165 170 175

Thr Tyr Arg Ser Lys Lys Pro Ala Lys Asn Leu Lys Met Pro Gly Val

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Tyr Tyr Val Asp Tyr Arg Leu Glu Arg Ile Lys Glu Ala Asn Asn Glu

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His Tyr Asp Ala Glu Val Lys Thr Thr Tyr Lys Ala Lys Lys Pro Val
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Arg His Phe Ala Ser Ala Ala Leu Pro Ile Pro Glu Val Leu Asp Ile
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Gln Pro Val Ala Glu Ala Met Asp Ala Ile Ala Ala Ala Asp Leu Ser
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His Trp Gln Thr Val Met Asp Asp Thr Val Ser Ala Ser Val Ala Gln
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Val Phe Glu Tyr Cys Leu Glu Asp Gly Ser Leu Ile Arg Ala Thr Lys
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His Leu Val His Ala Asp Phe Gly Ser Asn Asn Val Leu Thr Asp Asn
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Ala Leu Lys Asn Gly Phe Ile Ala Ser Asn Cys Met Glu Gln Gln Thr
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Arg Tyr Phe Glu Arg Arg His Pro Glu Leu Ala Gly Ser Pro Arg Leu

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Arg Ala Tyr Met Leu Arg Ile Gly Leu Asp Gln Leu Tyr Gln Ser Leu
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Val Asp Gly Asn Phe Asp Asp Ala Ala Trp Ala Gln Gly Arg Cys Asp
115 120 125
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